VOLUME 97 NUMBER 1

THE
BOTANICAL GAZETTE

*September 1935*

# STUDIES IN THE PHYLOGENY OF THE BETULACEAE[1]

## I. FLORAL AND INFLORESCENCE ANATOMY AND MORPHOLOGY

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(WITH TWO HUNDRED NINETY-EIGHT FIGURES)

### Introduction

The vexed question of the disposition of the Amentiferae in the phylogenetic classifications of today has led to this study of the Betulaceae. It is especially significant, in view of the importance attached to floral and inflorescence characters in present-day phylogenetic studies, to attempt to clarify the concept of the ament as it exists in this family. While much may be learned in this connection from a comparison of external structures alone, it is desirable to supplement this by a comparative study of the vascular anatomy of the florets and of the inflorescences, since it has been convincingly demonstrated for other groups of plants that the vascular system usually responds but slowly to changes in the external form of the floral organs.

But it is felt that, helpful as it may be in clarifying the morphology of the organ under consideration, a comparative study of the vascular systems of reproductive organs alone does not provide a sufficiently broad foundation for indicating interrelationships between groups of plants. In an attempt to supply this extra evidence for the Betulaceae, a study of the secondary xylem anatomy

[1] Contribution from the Laboratory of Plant Morphology, Harvard University.

of many of the species has been made, the precipitation reactions have been studied (**6**), and the observations of others in the fields of chromosome number and behavior and pollen morphology have been evaluated. These supplementary fields of evidence will be considered in a later paper.

It is felt that by establishing working hypotheses for the interpretation of the evidence from each of these fields, and then combining the results on the basis of the multiple working hypothesis (**5**), conclusions can be reached which would provide a basis for evaluating the phylogenetic positon of the Betulaceae. While the need of experimental verification of hypotheses and conclusions is strongly felt, the means of supplying this lack are not yet clear. Furthermore, a firm foundation should first be laid in comparative morphology before such means of verification are put into practice.

## Methods and materials

Herbarium material, when used, has been softened by immersion for several days in water kept warm on a warming table. A great majority of the studies are based on freshly collected specimens preserved in 70 per cent ethyl alcohol, a developmental series from several individuals of each species often being studied. Preparatory to study, a catkin is transferred to distilled water, allowed to soften, and with the usual instruments carefully dissected under water in a dish lined with beeswax. A pair of iridectomy scissors has been found helpful for the more delicate work. The better dissections have been preserved by mounting on slides in MASSART'S medium (**18**), while the best were drawn and later sectioned. Specimens were run up into paraffin (m.p. 56°–58° C.) through a slightly modified N-butyl alcohol series (**38**). Prior to this it was found necessary to soften all pistillate material with commercial hydrofluoric acid, full strength, for varying periods of time depending on the extent of sclerenchymatization encountered. Complete serial sections, 10–12$\mu$ thick, were made by means of a Spencer rotary microtome. The sections were stained on the slide with crystal violet and erythrosin (**17**).

While most of the specimens have been collected personally at the Arnold Arboretum, Harvard University, valuable aid has been

provided by Dr. E. ANDERSON, Dr. R. H. WOODWORTH, and DR. C. KOBUSKI of Harvard University. Except for the collections made by the latter two, these are authenticated by herbarium specimens. Material has also been obtained from Kew Gardens, England, on two occasions, and in the second case is identified by herbarium specimens. Further collections have been made by the writer on the north shore of the Gulf of St. Lawrence, in northern British Columbia and Alberta, in the Kaumajet and Torngat regions of Labrador, in North Carolina, in Norway, and in the vicinity of Ithaca, New York. Specimens have also been supplied from northern Canada by the late Dr. M. O. MALTE, from Greenland by Dr. M. P. PORSILD, from the state of Washington by Dr. W. C. MUENSCHER, from southern Labrador by Dr. H. F. LEWIS, and from China by Dr. A. N. STEWARD. Species which could not be obtained fresh were studied as described, from herbarium specimens kindly supplied by the herbaria at the Arnold Arboretum of Harvard University, at the Botany Department of Cornell University, and at the Botanical Garden, Berlin-Dahlem.

Identification of the material is based on the herbarium specimens or from field determinations. For checking the identifications, the writer is much indebted to Professor ALFRED REHDER of the Arnold Arboretum. The species names used are thus in general those to be found in his Manual of Cultivated Trees and Shrubs (**25**). Sets of herbarium specimens representing the material are to be found at the Arnold Arboretum and at Cornell University. The terms for the various subdivisions of the family and of its genera are in accord with the usage of WINKLER (**35**).

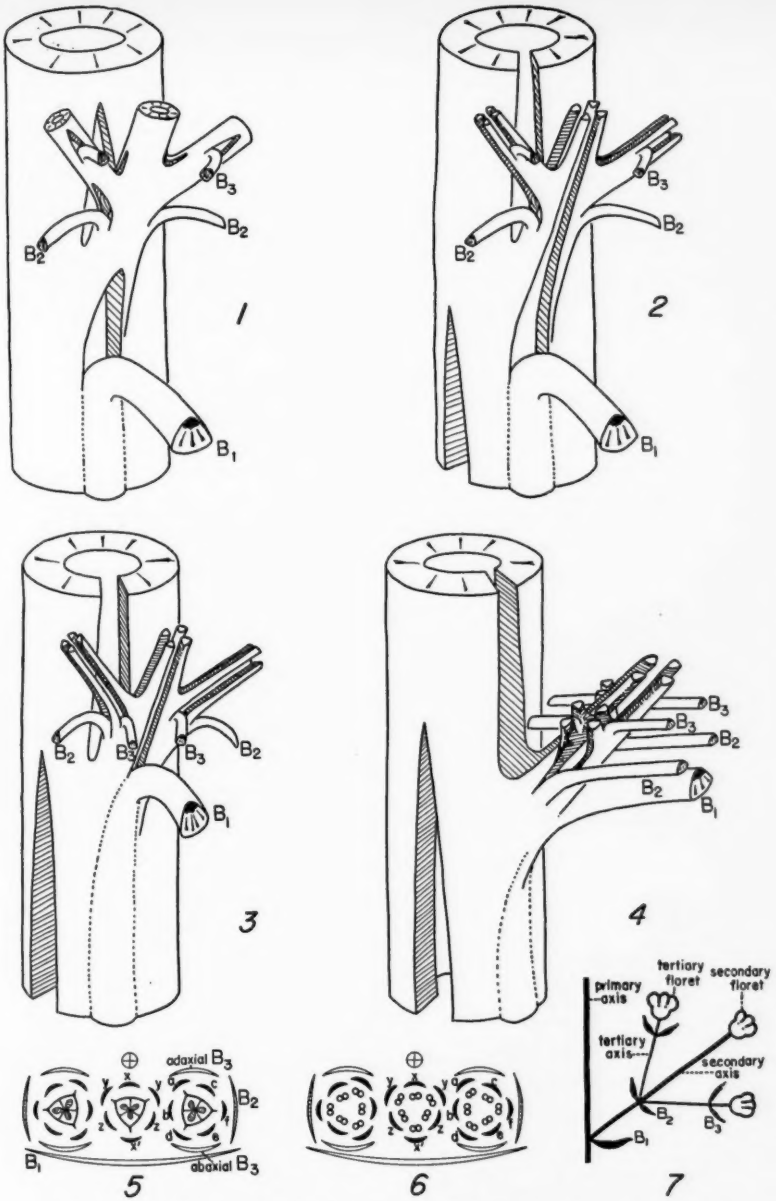
## Terminology

The condensed nature of the units of which the aments in this family are composed makes it difficult to assign them to any of the more general categories of inflorescence types. The extreme shortness of the pedicels of the individual florets brings these florets together so closely that they appear to form a diminutive cyme, or cymule. This latter term has been adopted, with the reservation that basically these lateral members of the inflorescence may be racemose since they are racemosely arranged on the ament axis.

The bracts attached to the primary axis of the ament are referred to as primary bracts ($B_1$) throughout; in their axils occur the secondary axes bearing secondary bracts ($B_2$); and in the axils of these occur tertiary axes bearing tertiary bracts ($B_3$). The secondary and tertiary axes are considered to terminate in secondary and tertiary florets respectively (fig. 7). Since the members of the floral envelope, the tepals, are not differentiated from one another, they are referred to collectively as the perigon. It is felt, for reasons which will be taken up in another place, that the pistils of the Betuleae (*Betula* and *Alnus*) are reduced inferior ovaries, and therefore the term floret will be applied to these as it is to their homologues in the Coryleae (*Carpinus, Ostrya, Corylus, Ostryopsis*).

**Cymule morphology and anatomy**

It is clear from the anatomy of the cymules of the 64 species, varieties, and hybrids examined that the simplest basis for description is the type of anatomical system associated with a unilacunar gap system. It is true that the foliage leaves are trilacunar (**28**), but there is a simple transition from the trilacunar foliage leaves to the unilacunar bracts of the inflorescence. In figures 1–4 an attempt is made to show the relationship of some of the simpler types of vascularization in the cymules of the Betulaceae to the more common conditions in vascular systems. In figure 1 the simplest condition is shown, where the gaps left by all of the bract traces close after giving rise from their sides to the two branch traces which later coalesce to form the siphonostelic supply to the floral pedicels. In figure 2 is shown the result of failure of the gaps of the secondary and tertiary bracts to close and of the branch traces to coalesce, so that there is a dissected siphonostele in the pedicels. With concrescence of the bracts to their axillary branches the bract traces depart tardily from the bases of their respective gaps; and, if there is superimposed on this a shortening of the internodes, the bract traces are brought into a more intimate relationship with each other (fig. 3). This latter condition is closely approximated in proliferated pistillate aments to be described under *Alnus crispa*. When this system comes under the influence of lateral and dorsiventral pressures, comparable to those in the aments of Betulaceae, then a side




FIGS. 1–7.—Figs. 1–4, vascular system with unilacunar gaps in cymule at successive stages of reduction: 1, vascular system in which the gaps close; 2, same in which the gaps persist; 3, same in which concrescence and shortening of internodes modifies relative positions of bract bundles; 4, lateral view of vascular system shown in previous figure under the influence of dorsiventral and lateral pressures. Figs. 5–7, diagrams to show relationship of parts and terminology employed in the cymule: 5, floral diagram of pistillate cymule with trimerous florets; 6, same of staminate cymule; 7, schematic diagram of cymule.

view of the vascular system would appear as shown in figure 4. Here the courses of the vascular bundles to the bracts become nearly parallel and the pedicellar supplies of the individual florets appear to diverge dorsally from this aggregate of bract traces. A careful investigation of figure 4, however, will show that the traces to the individual florets take their origin from the sides of gaps left in the dissected vascular cylinders by their respective subtending bract bundles. Conditions closely comparable to this exist in the typical cymule in *A. crispa*. Throughout the family the vascular system found in the cymules of all species is patterned after that in *A. crispa*.

## PISTILLATE CYMULES: MORPHOLOGY AND ANATOMY

### *Alnus, pistillate cymules*

The pistillate catkin in the species studied[2] throughout the genus is composed of a great number of cymules arranged helically on the primary ament axis. In most of the cymules (except those at the distal end of the ament which are much reduced) there is a primary bract, the secondary bracts, and the abaxial[3] tertiary bracts. Very rarely an adaxial tertiary bract develops, but is generally much reduced (fig. 64). Tertiary florets are uniformly present, but the secondary floret commonly develops only in proliferations. One occurrence of quaternary florets has been found, but a consideration of this is reserved for the next paper of this series.

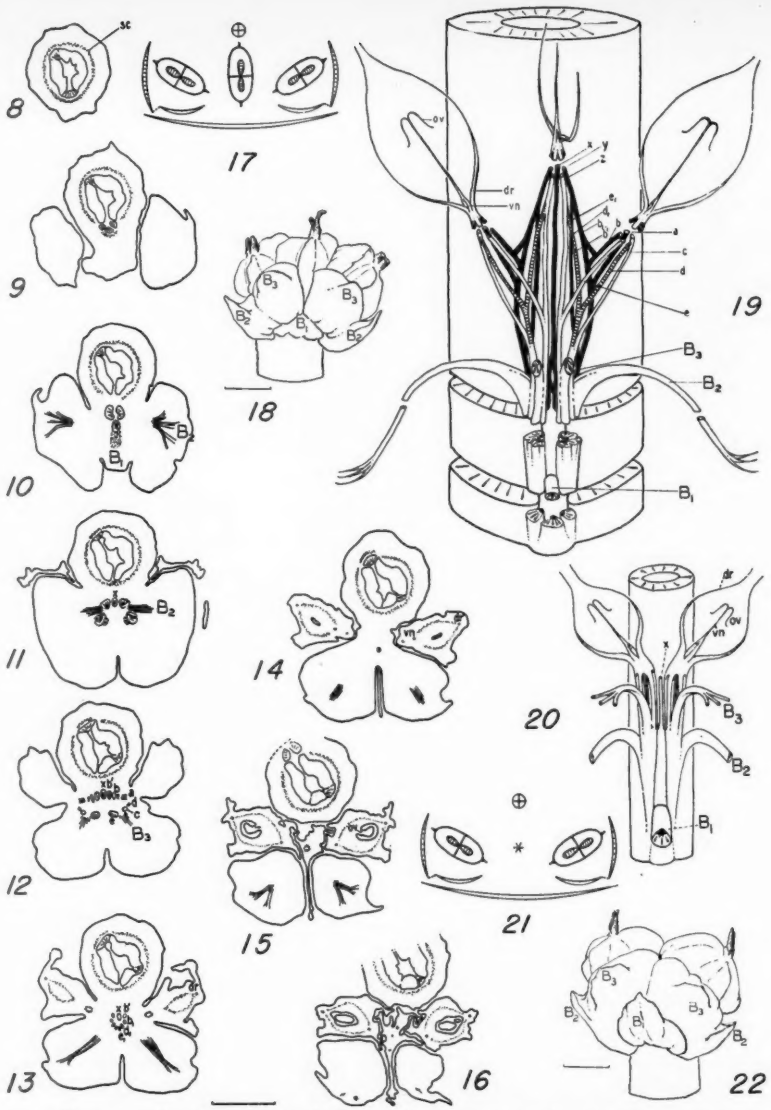
Because of the presence of a secondary floret in certain of the cymules of the proliferated material already mentioned, this makes a good starting point for a description of the vascular system of the cymule of the Betulaceae. Other than the presence of an occasional three-flowered cymule, this proliferated material of *Alnus crispa* differs from the typical condition in the elongation of the lower

[2] Section Alnobetula: *A. firma* var. *hirtella*, *A. crispa*, *A. crispa* var. *mollis*.
Section Gymnothyrsus: *A. subcordata*, *A. maritima*, *A. japonica*, *A. rugosa*, *A. incana*, *A. hirsuta*, *A. rubra*, *A. tenuifolia*, *A. jorullensis*, *A. spaethii*.
Section Clethropsis: *A. nitida*, *A. nepalensis*.
Section Cremastogyne: *A. cremastogyne*, *A. lanata*.

[3] The terms abaxial and adaxial are used in relation to the primary axis of the ament. cf. floral diagram in figure 5.

internodes of the ament to such an extent that the ordinary dorsiventral pressure is removed. Thus there is a corresponding tendency toward a more normal orientation of the component parts of the cymules in this region. Above the region of marked proliferation the cymules have the morphology of those of ordinary catkins (fig. 40). A three-flowered cymule from the transition region is shown in figure 18 and a two-flowered one in figure 22.

The vascular systems of two- (fig. 20) and three-flowered (fig. 19) cymules show striking resemblances to those constructed on a purely theoretical basis in figures 1–4. Especially is this so for the three-flowered cymule. Starting well down in the primary axis of the ament (fig. 8), the first evidence of a vascular supply to the three-flowered cymule under consideration (cf. fig. 19 for reconstruction of the vascular system) appears as a wedge of xylem demarcated from the rest of the vascular system of the catkin axis by rather strong rays on either side. This wedge of vascular tissue soon departs from the main siphonostele, leaving a gap in the latter, and divides into three parts (fig. 9). The central one continues without further complications into the primary bract, while the two lateral bundles form arcs (fig. 10) which coalesce loosely to form the vascular cylinder with a gap in the region of the tardily departing primary bract of the secondary axis. No sooner is the cylinder of the secondary axis formed than the traces to the secondary bracts differentiate (fig. 11) and swing out and down (fig. 10), leaving a gap on either side of the vascular cylinder of the secondary axis. From the sides of these gaps depart a series of bundles, which bifurcate successively, one branch passing to the tertiary floret and the other branch remaining to supply the secondary floret. Thus bundles *a*, *b*, *c*, *d*, and *e* pass off laterally to form the vascular system of the tertiary axis and pedicel, while their homologues in the secondary axis fuse in pairs to form four of the traces (*y*, *y*, and *z*, *z*, fig. 19) in the pentamerous dictyostele. While this departure of the bundles to supply the tertiary axis is going on, the bundle to the abaxial tertiary bract also departs, leaving a gap from the sides of which are derived the bundles *d* and *e*. In addition to the homologues of the traces of the tertiary florets, there is another bundle in the secondary axis which is unique (*x*, fig. 19).

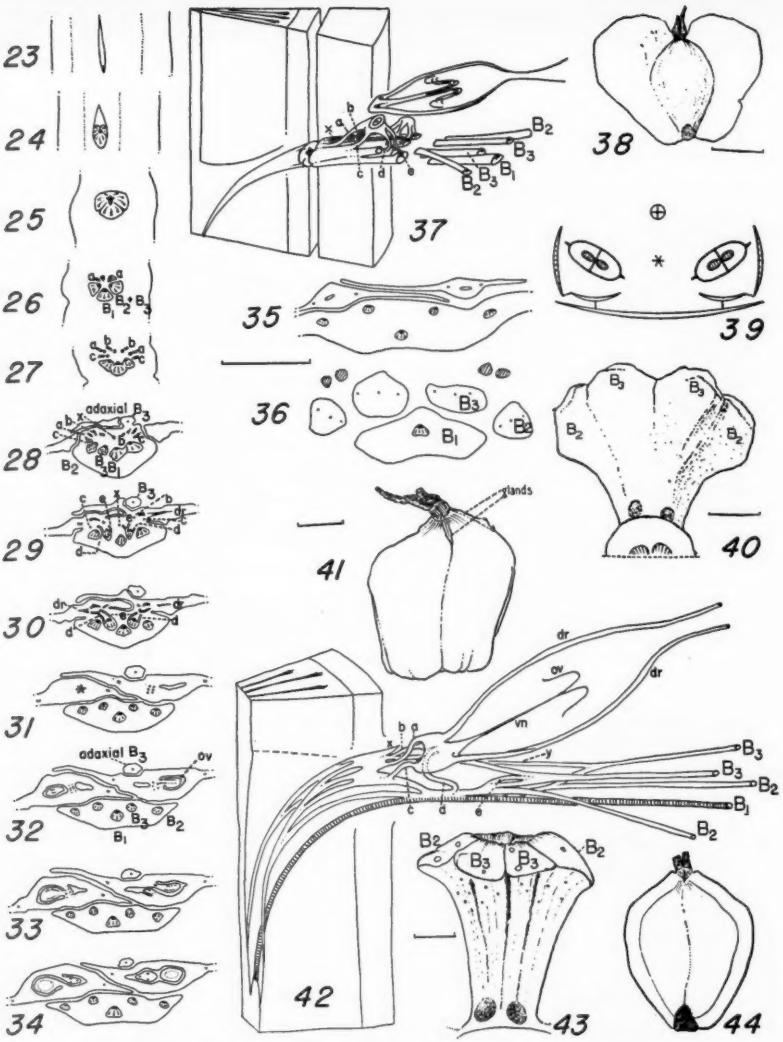



FIGS. 8–22.*—*Alnus crispa*, pistillate. Figs. 8–16, successive transverse sections through cymule shown in fig. 18; fig. 17, floral diagram of cymule shown in following figure; fig. 18, three-flowered cymule from base of proliferated ament; fig. 19, reconstruction of vascular system of cymule shown in preceding figure; fig. 20, same of cymule shown in fig. 22; fig. 21, floral diagram of cymule shown in following figure; fig. 22, two-flowered cymule from base of proliferated ament.

* Serial sections so arranged that they proceed distally through cymule. The scale of the figures is shown by a horizontal line near them representing 1 mm. Reconstructions are not drawn to scale and are sometimes exaggerated in the pedicellar region to show distribution of vascular tissue to better advantage. Pubescence and glands omitted in most cases from habit sketches. Single index letters used for vascular bundles or for the organs which they supply are in accord with usage indicated in floral diagrams, figs. 5 and 6. Bundles to bracts indicated by the abbreviations for primary ($B_1$), secondary ($B_2$), and tertiary ($B_3$) bracts. Other abbreviations used for structures found associated with the cymules are: *anas*, anastomosis; *dr*, dorsal trace; *invol*, involucre; *lam*, lamina; *lc* or *loc*, loculus; *ov*, ovule trace; *pd*, pedicel or pedicellar vascular system; *pg*, perigon; *sc*, sclerenchyma; *st*, stamen; *stip*, stipule; *vn*, ventral ovary trace.

The vascular system (fig. 20) of the two-flowered cymules (fig. 22) is essentially similar to that already described, except that in the absence of the secondary floret there is a corresponding absence of vascular tissue in the secondary axis, except for bundle *x*, which sometimes remains independent. Ordinarily *x* swings to one side or the other and fuses with one of the nearby traces, a behavior characteristic of the vascular supply to degenerate or recently lost organs.

The condition just described provides a convenient transitional stage to that of the vascular system (fig. 37) of a typical cymule in a variety of this species (*A. crispa* var. *mollis*). Figure 40 represents the external morphology of a cymule with the two florets removed, while figure 37 shows a reconstruction of the vascular system of such a cymule. The vascular system originates as a small number of protoxylem elements swinging out through the vascular cylinder of the primary axis and forming an elongate gap (fig. 23). As the xylem of the bundle progresses farther through the vascular cylinder of the primary axis, the gap becomes broader and the bundle accumulates more secondary xylem (fig. 24). By a lateral overarching, this bundle becomes roughly cylindrical in the cortex of the ament axis (fig. 25). At this level it represents the vascular cylinder of the secondary axis accompanied by the trace to the primary bract. The primary bract bundle soon becomes free (fig. 26) and, except for temporary anastomoses, continues independently into the primary bract. On either side of this primary bract trace there differentiates a large bundle, triangular in section (fig. 26), which splits to form two (fig. 28). The outer of these is the bundle to the secondary bract and the inner the bundle to the tertiary bract. The small traces (*a*, *b*, *c*, *d*, *e*) departing from the flanks of these large bundles correspond to the same traces described in the proliferated material (fig. 19), and are actually derived from the sides of the gaps left by the departure of the large bract bundles. Thus they represent the vascular system of the tertiary axes. So short a distance do they traverse before their reorganization in the bases of the florets, however, that this reorganization has already begun on one side of the pedicel while the bundles are still arising on the other side (figs. 29, 30). The trace *x*, which was first met in the proliferated material, is present as a weak bundle which later coalesces with the nearby trace *e*.




FIGS. 23–44.—Figs. 23–40, *Alnus crispa* var. *mollis*, pistillate: figs. 23–36, successive transverse sections through cymule similar to that shown in fig. 40; fig. 37, reconstruction of vascular system of cymule shown in preceding figures; fig. 38, single floret shown from side of attachment; fig. 39, floral diagram of representative cymule; fig. 40, cymule with florets removed. Fig. 41, *A. subcordata*, single floret to show glands indicative of vestigial perigon. Figs. 42–44, *A. nitida*, pistillate: fig. 42, reconstruction of course of primary xylem in vascular system of cymule; fig. 43, cymule with florets removed; fig. 44, single floret viewed from side of attachment.

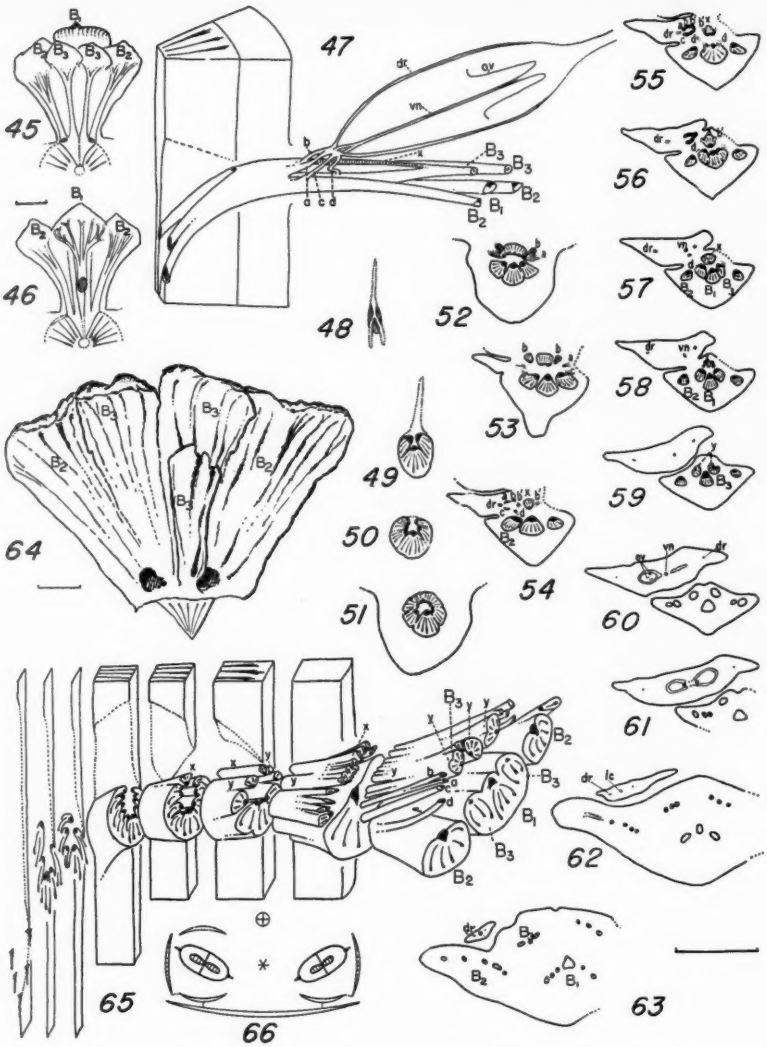
Interestingly enough, there was found in the example chosen for sectioning a small, free, flattened structure opposite one of the tertiary florets. This is probably the adaxial tertiary bract.

An essentially identical distribution of vascular tissue exists in the other species studied in the section Alnobetula.

Representative of the section Gymnothyrsus is *Alnus incana* (figs. 45–63). The morphology of the cymule (figs. 45, 46) is essentially the same as that in the preceding section of the genus. In the vascularization of the cymules (fig. 47), however, there are some slight differences. More notable among these is the tripartite nature of the origin of the vascular supply to a cymule (fig. 48). This approaches much more closely to the vascular condition in the proliferated material of *A. crispa* than to the typical material of that species. Each of the three bundles accumulates secondary xylem in its passage outward (figs. 49, 50) into the cortex and there forms a rough siphonostele (fig. 51). In the course of the close association of the primary bract bundle with the adjacent vascular tissue, the vascular supply to the tertiary bracts remains with that to the primary bract rather than with that to the secondary, as in *A. crispa*. A better defined trace *x* is present here than in the normal cymules of the s.[4] Alnobetula. As a further novelty, the trace *x* splits (fig. 58) and either half (*y* and *y*) swings downward to fuse (fig. 59) with the tertiary bract bundles after they become free from the primary bract bundle.

The relationships of the cymule bundles are essentially the same as described in s. Alnobetula, except for the absence of the trace *e*, simplifying the supply to the tertiary florets. There is clearly a correlation between the absence of the trace *e* and the prolonged association between the primary and tertiary bract bundles. As a result the trace *e* is not liberated in time to make connections with the vascular system of the tertiary axis. Instead it accompanies the tertiary bract bundles in their fusion with the traces *y* and *y* (fig. 59). In other species of the s. Gymnothyrsus this intermediate behavior of the trace *e* is more pronounced, showing the intimate structural similarity between the members of the two subgenera. Mention has already been made of the presence of an adaxial tertiary bract

[4] Throughout the following pages, section is abbreviated as s.; subsection as ss.




FIGS. 45–66.—Figs. 45–63, *Alnus incana*, pistillate: figs. 45, 46, upper and lower sides respectively of young cymule with florets removed and vascular system dissected out; fig. 47, reconstruction of vascular system shown in following figures; figs. 48–63, successive transverse sections of cymule similar to fig. 45. Figs. 64–66, *A. lanata*, pistillate: fig. 64, upper surface of mature cymule with florets removed (note presence of adaxial tertiary bract); fig. 65, reconstruction of vascular system of basal portion shown in preceding figure; fig. 66, floral diagram of cymule shown in fig. 64.

in *A. crispa* var. *mollis*. Examples of this condition were found also in *A. lanata* of s. Cremastogyne (fig. 64). The vascular system (fig. 65) of this cymule shows that the origin of the bundle to the adaxial tertiary bract is exactly as might be expected, since it is derived from the position corresponding to the origin of the abaxial tertiary bract, opposite. The bundles *y* and *y* on either side of the adaxial tertiary bract trace, the bundles *a*, *b*, *c*, *d*, and the abaxial tertiary bract trace, represent the vascular system of the tertiary axis. In this system the adaxial and the abaxial tertiary bract bundles occupy diametrically opposite positions, and lie in a plane at right angles to that of the departure of the bundle to the bract of the second order.

In the species of s. Clethropsis, as shown for *A. nitida* (fig. 42), the vascular system originates as it does in *A. incana* (fig. 48), but the branch traces quickly subdivide so that there is a supply of at least six discrete bundles to the cymule, with the bundle *x* also often becoming separate. This characteristic is peculiar to the two species of this section and to the two species of s. Cremastogyne (fig. 65) which do not differ greatly in pistillate morphology and anatomy from those of s. Clethropsis. The origin of the tertiary vascular system is much the same as that in s. Gymnothyrsus. There is also a similar behavior of the trace *e* of this axis to that found in some species of s. Gymnothyrsus. Departing so tardily from the upper side of the tertiary bract trace that it cannot serve in the formation of the tertiary vascular axis, trace *e* fuses with the remnant of bundle *y* (fig. 42) at the time of the anastomosis of that bundle and the tertiary bract bundle. Of further interest in *A. nitida* is the sporadic occurrence of three-flowered cymules, their vascularization indicating that the supernumerary floret is indeed the secondary floret. These supernumerary florets occur in otherwise normal, non-proliferated aments.

In brief, the vascularization of the pistillate cymules of *Alnus* supports the generally current view that here the inflorescence was originally three-flowered (or possibly more) while the adaxial tertiary bracts have been lost by reduction. Furthermore, it is evident that the vascular system is not far removed from less modified conditions such as were discussed in relation to figures 1–4. Essentially,

figure 4 provides a schematic representation of the vascular system in most species of the genus. It is evident that the simple relationship of bract trace to branch traces is the basic feature in the organization of vascular system of the cymule in this genus.

*Betula, pistillate cymules*

The pistillate cymule of the species studied[5] in this genus generally has all three florets present, although only the secondary floret is present in some species of the ss. Nanae. The bracts of the third order are consistently absent, and among the species of ss. Acuminatae and ss. Nanae there is a tendency toward the loss of the secondary bracts.

There is great similarity in the origin and course of the vascular bundles supplying the cymules of all the species studied in the ss. Costatae. This may be attributed to two factors. One is the relative simplicity of the vascular supply contrasted with that of the cymules in *Alnus*, and the second is the presence of only primary xylem in the critical basal region of the cymule. This latter condition is doubtless correlated with the generally undifferentiated nature of the tissues in that region of the cymule, because of the dehiscence of the cymules from the primary axis of the inflorescence soon after the fruit matures.

As in many species of *Alnus*, the vascular system (fig. 67) of the cymule of *B. lenta* originates as three separate groups of primary xylem (fig. 68), the one at the base of the gap being the bract trace, the other two (the laterals) being the branch traces representing the

[5] Section Eubetula

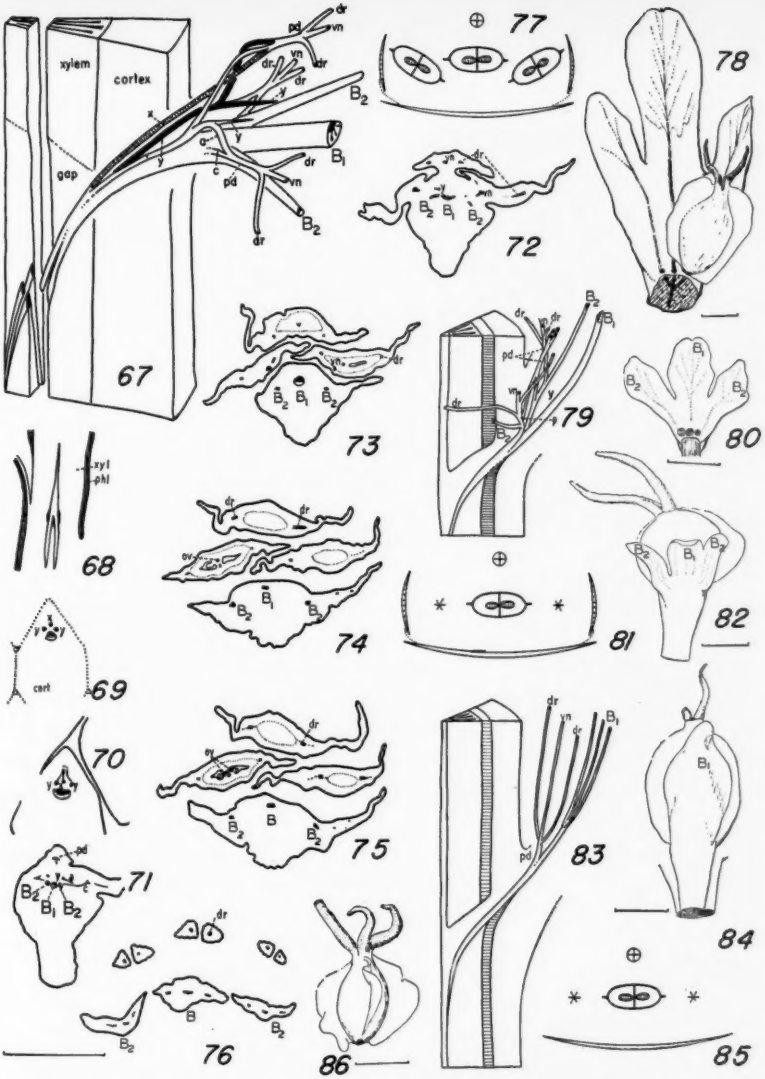
Subsection Costatae: *B. schmidtii*, *B. globispica*, *B. lenta*, *B. grossa*, *B. utilis* var. *pratii*, *B. lutea*, *B. medwediewi*, *B. nigra*.

Subsection Albae: *B. papyrifera* var. *subcordata*, *B. papyrifera* var. *occidentalis*, *B. papyrifera* var. *carpatica*, *B. coerulea-grandis*, *B. pendula*, *B. japonica* var. *mandshurica*, *B. populifolia*, *B. davurica*.

Subsection Nanae: *B. michauxii*, *B. glandulosa*, *B. pumila*, *B. nana*.

Section Betulaster

Subsection Acuminatae: *B. luminifera*, *B. alnoides* var. *pyrifolia*, and *B. maximowicziana* (probably hybrid material).




FIGS. 67–86.—Figs. 67–77, *Betula lenta*, pistillate: fig. 67, reconstruction of vascular system of cymule in section in following figures; figs. 68–76, successive transverse sections; fig. 77, floral diagram representative of preceding figures and of fig. 78. Fig. 78, *B. medwediewi*, pistillate cymule with two of the three florets removed. Figs. 79–82, *B. nana*, pistillate: fig. 79, reconstruction of vascular system of cymule similar to that shown in following figure; fig. 80, cymule with florets removed; fig. 81, floral diagram for cymule shown in following figure; fig. 82, one-flowered cymule seen from lower side of bract complex. Figs. 83–85, *B. michauxii*, pistillate: fig. 83, reconstruction of vascular system of cymule similar to that shown in following figure; fig. 84, mature cymule seen from lower side; fig. 85, floral diagram of cymule shown in preceding figure. Fig. 86, *B. luminifera*, tricarpellary ovary.

secondary axis. This is the vascular plan characterizing the inception of an axillary branch and its subtending leaf. A very weak cylinder forms from these three bundles; in the cortex this breaks up into four bundles (fig. 69). The uppermost of these four (*x*) continues into the secondary floret while the two lateral bundles (*y* and *y*) bifurcate, each sending one branch to the secondary floret and the other branch (*a*) to the nearest tertiary floret (figs. 69–71). In the origin of the traces destined to vascularize the tertiary floret the large lower median bundle gives off on either side a large lateral branch from whose flanks small bundles (*c* and *c*) pass to the nearest tertiary floret, to take part in the formation of the pedicellar supply (fig. 71 *et seq.*). Ultimately these large lateral bundles form the supply of the secondary bracts (fig. 76). After they contribute vascular tissue to the tertiary florets, the traces *y* and *y* persist for a short time, suggesting a possible tertiary bract supply.

Evidently there is a strong homology between the vascular system of *Betula* and *Alnus*. Whereas in *Alnus* the poorest development of the vascular supply to the tertiary floret still consisted of four bundles (*a*, *b*, *c*, *d*), there are in ss. Costatae of the genus *Betula* but two bundles (*a* and *c*), suggesting the formerly more complex condition. The secondary floret in this section is somewhat more richly supplied with vascular tissue (traces *x*, *y*, *y*) than are the tertiary florets. It is significant that the trace *x*, which was found to persist in *Alnus* when the secondary floret was absent, is also present in *Betula* and constitutes a major portion of the supply to the secondary floret. The other species of this subsection are essentially similar to *B. lenta*, but tend toward a further simplification of the vascular system, approaching that characteristic of the species in the next subsection.

Instead of the three separate traces to the cymule characteristic of most of the species of the preceding subsection, the species of ss. Albae have but a single trace as the origin for the vascular supplies of the primary bract and secondary axis. Apparently there is such a close apposition of the branch traces to the intermediate bract trace that, in the absence of secondary xylem, it is impracticable to distinguish them. The trace *x* is found to be lacking after the very weak cylinder formed earlier is broken up, and the second-

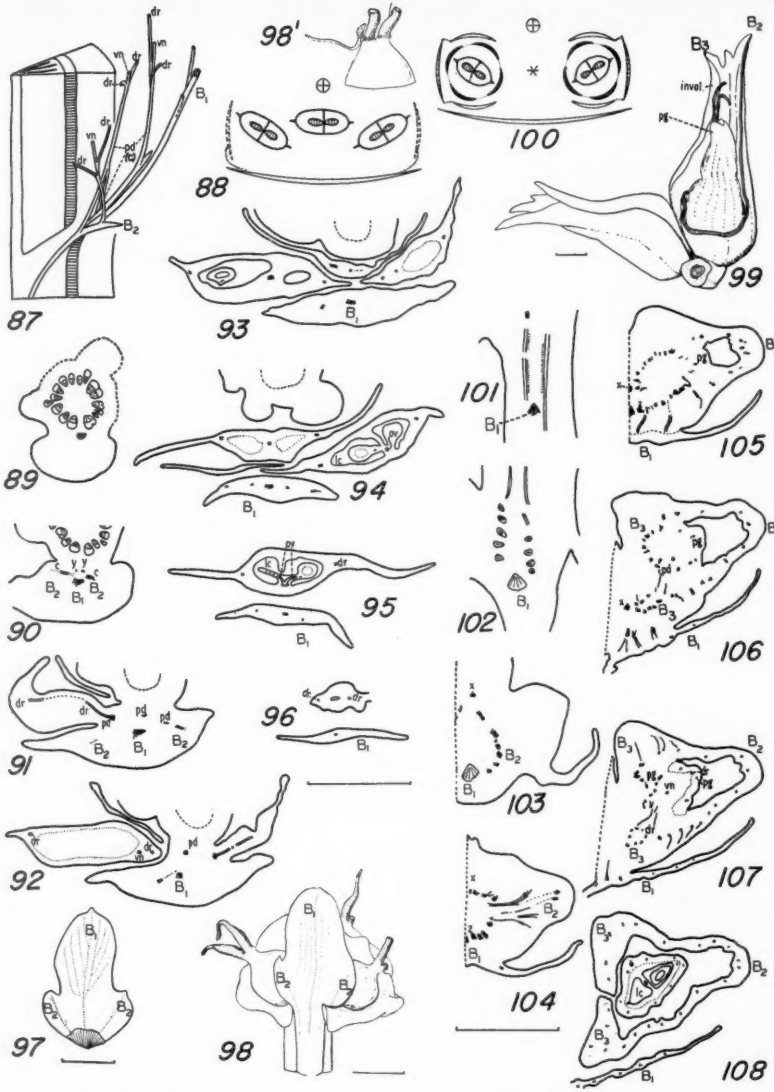
ary floret is supplied only by a branch from each of the traces *y* and *y*. A branch from each of the *y*'s then passes to the nearest tertiary floret and fuses with trace *c*, which has meanwhile split off from the secondary bract supply. The remainder of each of the *y*'s persists for but a short time. In all essentials the vascular system in the other species of ss. Albae is of this nature and represents a simplified version of the Costatae type.

Except for *B. michauxii* the species of ss. Nanae generally have three-flowered cymules, but in many cases entire aments may be composed of one-flowered cymules. In *B. michauxii* (fig. 84), not only are the cymules one-flowered but the secondary bracts are lacking, thus reducing the cymule to the ultimate in simplicity.

The vascular system of *B. nana* (fig. 79) exemplifies the condition in the three-flowered cymules of the species in this subsection. It is practically identical in vascularization with the cymules of ss. Albae, except for the absence of any prolongation of *y* beyond the departure of trace *a*, so that the two become indistinguishable. In the one-flowered cymules (fig. 82) of this species there is essentially this same vascular condition except that the tertiary florets and their vascular supplies fail to develop.

Finally in *B. michauxii* (fig. 84) no vascular supply (fig. 83) is even suggested for the tertiary florets or for the secondary bracts, while the single floret derives its vascular supply from a single branch of the single bundle to the primary bract. So simple is the inflorescence that the entire ament may be called a spike in the strictest sense of the term, although it is undoubtedly a spike by reduction, despite the fact that there are no vestigial traces to those parts of the cymules which are lost.

Of the species studied in ss. Acuminatae, *B. maximowicziana* stands as morphologically distinct, perhaps owing to the probable hybrid nature of the material. The vascular system is practically the same as that described for *B. lenta*, ss. Costatae. The other two species studied are of the greatest interest because they exhibit stages transitional to the apparent loss of the secondary bracts. In *B. luminifera* the cymules have the secondary bracts recognizable although reduced as are those shown for *B. alnoides* var. *pyrifolia* in figure 97. But in the latter there also occur more extreme conditions




FIGS. 87–108.—Figs. 87–96, *Betula luminifera*, pistillate: fig. 87, reconstruction of vascular system of cymule; fig. 88, floral diagram of cymule; figs. 89–96, successive transverse sections (*cf.* fig. 87). Figs. 97, 98, *B. alnoides* var. *pyrifolia*, abaxial and adaxial views of pistillate cymule. Fig. 98′, *B. coerulea-grandis*, upper portion of floret to show tepal gland. Figs. 99, 100, *Ostryopsis nobilis*, pistillate: fig. 99, cymule with portion of involucre cut away from around one floret; fig. 100, floral diagram of cymule. Figs. 101–108, *O. davidiana*, successive transverse sections through pistillate cymule similar to that shown in fig. 99.

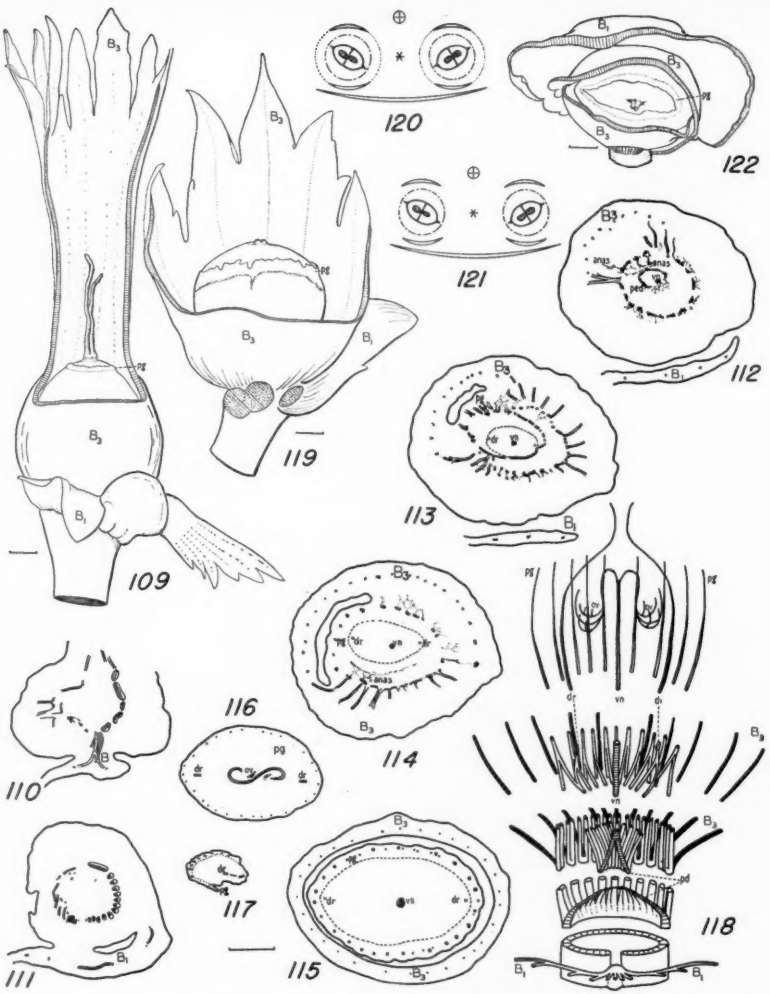
(fig. 98), where one of the secondary bracts has merged completely with the primary bract while the other is still distinguishable. Not illustrated but commonly present in the material are cymules in which both secondary bracts are fused completely with the primary bract and only the distinctness of their vascular systems remains to indicate the basic nature of the cymule bract system. The vascular system of the cymules of *B. luminifera* (fig. 87) differs from that of the Albae in that the traces *y* and *y* do not take part in the vascular supply of the secondary florets, which is therefore composed only of trace *c*. The latter is derived from the weak secondary bract trace, which soon disappears.

The vascularization of the cymules in *Betula* provides no example of the presence of vascular tissue which may have supplied tertiary bracts. The secondary bracts are present in practically all the species, but they are so reduced in two of the species of ss. Acuminatae that a parallel series doubtless accounts for their absence in *B. michauxii* of ss. Nanae. While the secondary floret is always present in the cymules of the genus, the absence of the tertiary florets in *B. michauxii* is directly correlated with the extreme reduction of the cymule and its vascular system.

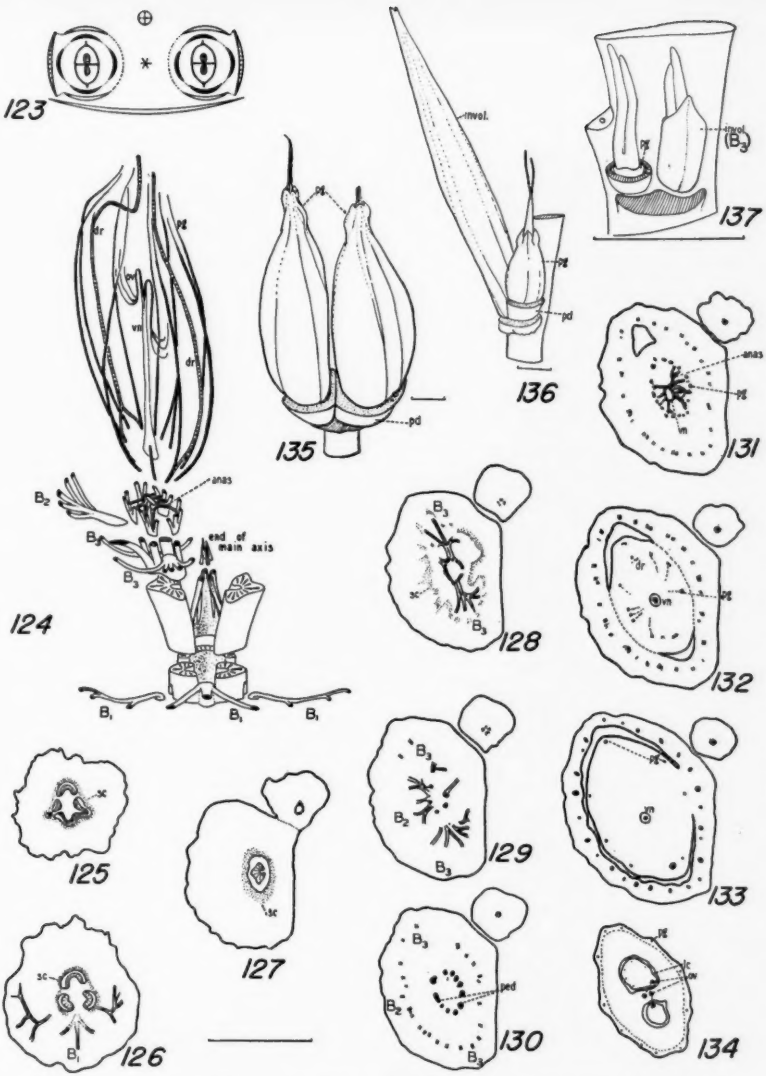
### *Carpinus, pistillate cymules*

Perhaps the most striking feature in the inflorescence morphology of the species studied[6] is the disposition of the involucre, which in s. Eucarpinus is plane, three-lobed, or (if at the base of the ament) with either or both of the tertiary bracts free from the secondary one, the secondary bract much exceeding the tertiary ones in size. In s. Distegocarpus the adaxial tertiary bract is free, while the abaxial one is often so completely fused with the secondary bract that its identity is clearly detectable only in the vascular system of the cymule. Important also is the length of the internodes of the pistillate cymules of *Carpinus* which results in less telescoping of the vascular system, making it easier to interpret than that in the genera considered previously.

[6] Section Distegocarpus: *C. cordata, C. japonica.*
Section Eucarpinus: *C. caroliniana, C. turczaninowii, C. laxiflora, C. orientalis, C. betulus.*




FIGS. 109–122.—Figs. 109–118, *Corylus cornuta*, pistillate: fig. 109, abaxial view of half-mature cymule with part of involucre cut away; figs. 110–117, successive transverse sections from base upward through cymule shown in fig. 109; fig. 118, reconstruction of vascular system of cymule shown in figs. 109–117. Figs. 119, 120, *C. maxima*, pistillate: fig. 119, lateral view of half-mature cymule, part of involucre cut away to show pistil, the other floret and cymules also removed; fig. 120, floral diagram of cymules shown in figs. 109 and 119. Figs. 121, 122, *C. heterophylla*, pistillate: fig. 121, floral diagram of cymule shown in following figure; fig. 122, view from above, smaller floret and other cymules of inflorescence having been removed as well as parts of involucral bracts to show upper portion of ovary.



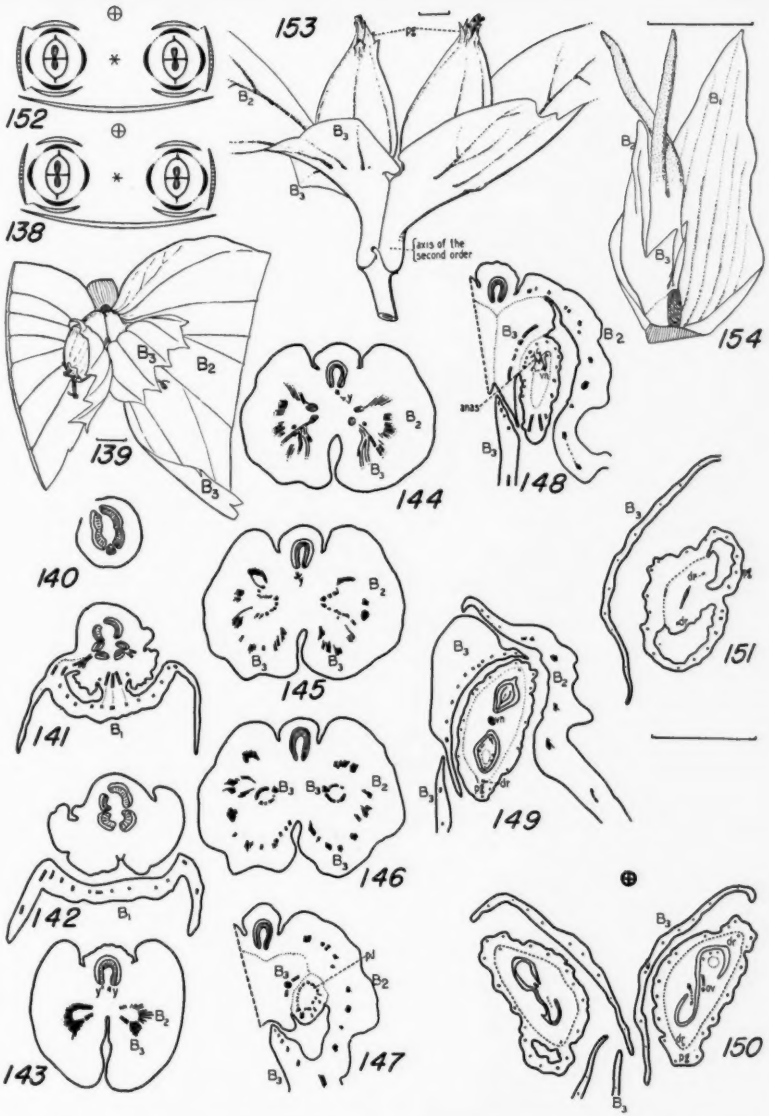

FIGS. 123–137.— Figs. 123–134, *Ostrya carpinifolia*, pistillate: fig. 123, floral diagram for *Ostrya* in general; fig. 124, reconstruction of vascular system of one-half of cymule, based on following figures; figs. 125–134, successive transverse sections through one member of a cymule (*cf.* reconstruction of vascular system in preceding figure). Figs. 135–137, *O. virginiana*, pistillate: fig. 135, cymule with involucre and primary bract completely removed; fig. 136, cymule in early stage of development with involucre cut away from one floret and primary bract removed; fig. 137, cymule at early stage of development with involucre from one floret and also primary bract removed.

*Carpinus japonica* has been chosen to illustrate the genus. So similar is it in the vascularization of the cymule to *Ostrya* that the reconstruction for the latter (fig. 124) will serve equally well here. The primary bract of the species studied in the s. Distegocarpus is trilacunar in its vascularization (fig. 141) while in s. Eucarpinus it is essentially unilacunar. There is a well defined secondary axis vascular system (fig. 142) which soon separates laterally (fig. 143) to make up the tertiary branches, leaving behind from their adaxial shoulders the traces *y* and *y* as the sole representatives of the secondary vascular system. After persisting to the level at which the bundle of the tertiary bract departs these finally disappear (fig. 145), a type of behavior also found in *Ostryopsis*. The organization of the tertiary vascular cylinders is soon followed by the differentiation of the secondary and abaxial tertiary bract bundles (fig. 143) which depart and immediately branch (fig. 144). There is left a poorly defined tertiary vascular axis from which is derived the bundle to the free adaxial tertiary bract (fig. 146). The apparent tardiness with which this occurs is occasioned by the tilt of the tertiary axis so that the sections are not truly transverse. It is also significant that the bundle to the free tertiary bract does not branch until it is well away from the tertiary vascular system. On the other hand, that of the abaxial tertiary bract branches as it departs from the vascular cylinder of the tertiary axis which contributes to the vascular system of the compound bract (composed of the primary and the secondary bract). This type of behavior indicates the influence of the "fusion" of organs on the behavior of their vascular systems.

*Ostrya, pistillate cymules*

The similarity to *Carpinus* of the species studied[7] in this genus is at once apparent. The major differences in external morphology are the shorter tertiary axes (*pd*, figs. 135, 136) and the lateral fusion of the tertiary bracts to each other as well as to the secondary bract to form an involucre (*invol*, fig. 137). In early developmental stages (fig. 136) in this genus the tertiary bracts are much more evident than is the secondary, while the reverse is the case in *Carpinus*

[7] *O. carpinifolia, O. japonica, O. knowltonii, O. virginiana.*




FIGS. 138–154.—Figs. 138–151, *Carpinus japonica*, pistillate: fig. 138, floral diagram for following figure; fig. 139, view of cymule from above, outer portions of primary bracts removed; figs. 140–151, successive transverse sections through bract-complex shown in preceding figure (in fig. 150 both ovaries are shown). Figs. 152–154, *C. caroliniana*, pistillate: fig. 152, floral diagram for following figures; fig. 153, adaxial view of cymule, outer ends of primary bracts removed; fig. 154, early developmental stage, one member of cymule removed.

(fig. 154). This discrepancy in size is reflected in the vascular system, and in the nearly mature involucre (fig. 136) in which the two tertiary bracts are indicated by the teeth in the margin of the involucre.

The vascular system of *O. carpinifolia* is shown in a three-dimensional reconstruction in figure 124, and has been chosen for description because it shows most clearly the basic relationships of the various organs and their vascularization. The vascular supply to the primary bract is trilacunar (figs. 125, 126), like that of *Carpinus japonica*, while the other species are more nearly comparable to *Ostryopsis* where there is only a suggestion of a multilacunar condition. Aside from this the species are very similar in the vascularization of their cymules, except that in *O. carpinifolia* the vascular supply to the secondary and tertiary bracts is more clearly defined. In the latter the branching of the vascular system occurs so close to the vascular axis that it is often difficult to determine the individuality of the bracts. Indeed the vascular system of the secondary bract shows a strong tendency in the other species to unite with that of the adjacent tertiary bracts, suggesting the functional abortion of the secondary bract and indicating a parallelism to the situation to be considered later in *Corylus*. The length of the secondary and tertiary axes is sufficient for these to show definitely organized vascular systems. Thus there is a secondary vascular system present, at the level shown in figure 126, composed of two arcs of well developed vascular tissue. The two arcs swing laterally, leaving no vestigial tissue representative of the secondary axis, and each becomes the vascular tissue of a tertiary axis (fig. 127 shows one of the two tertiary axes in section). From the tertiary vascular system the bundles to the tertiary bracts soon depart (fig. 129). Owing to concrescence, the vascular supply to the secondary bract is tardy in its departure (fig. 129) from its prolonged association with the tertiary vascular axis. The bundles to the secondary and tertiary bracts branch almost immediately to form the many small bundles of the main portion of the involucre (fig. 129 *et seq.*). The vascular tissue remaining after the departure of the secondary and tertiary bracts is in the form of a loose dictyostele from which the floral supply is later derived.

The essentially simple nature of the vascularization of the cymule here and in *Carpinus* accords well with the hypothesis established somewhat earlier in this paper, namely, that the cymule of the Betulaceae possesses a vascular system which is fundamentally characterized by the simple relationships of bract to axillary branch slightly modified by concrescence and shortening of the internodes.

*Ostryopsis, pistillate cymules*

*O. davidiana* and *O. nobilis* in this genus were studied and found to be practically identical in their morphology and vascularization. In both species the cymules possess only the tertiary florets. The cymules are well separated from each other on the primary axis so that there are absent the dorsiventral and lateral pressures which so modify the form of the cymules in *Alnus* and *Betula*. There are but few cymules in the catkin as well. The primary bract is free from the other bracts. Lateral fusion of the secondary to the tertiary bracts (which are of about the same length) has resulted in the formation of an involucre (fig. 99). *Ostryopsis* differs from *Carpinus* and *Ostrya* further in the shortness of the tertiary axes of the cymules, resulting in a practically sessile condition of the tertiary florets.

Like the vascular supply to the cymules of the other genera described, there is formed first in the vascular cylinder of the primary axis of the ament a gap (fig. 101) with a large bundle at the base which proceeds directly into the primary bract. From the sides of the gap arise a series of traces (fig. 101) which form a dictyostelic, laterally compressed cylinder in the cortex of the primary axis (fig. 102), and from which there departs the broad bundle to the secondary bract (fig. 104) in a plane at right angles to that of the primary bract bundle. The numerous small bundles flanking this region of departure reorganize to form the tertiary vascular cylinder (fig. 105) from which very soon there depart the bundles to the tertiary bracts in a plane parallel with that of the primary bract and at right angles to that of the secondary bract. Bundles $x$ and $z$ (figs. 103–105) representing the secondary vascular axis ultimately disappear without taking part in the supply to any of the organs present in the cymule. Several accessory traces pass to the primary bract. This may represent amplification resulting from the working

back of the primary bract traces into the vascular system of the secondary axis, a process possibly associated with concrescence of the primary with the secondary axis.

*Corylus, pistillate cymules*

As in *Ostryopsis*, the species studied[8] in this genus have but few cymules in an ament, but, unlike *Ostryopsis*, these cymules are closely crowded, and only a small proportion of them mature fruit. Often in the uncultivated species and varieties it is only one of the tertiary florets which develops in a given cymule while the other floret has been arrested early (fig. 109). The successful floret then, because of its vigorous growth, deflects the smaller floret and in many cases appears to terminate the primary axis of the ament. The species studied provide a series of steps from complete freedom of the bracts of the third order to their lateral fusion and modification to the form of a campanulate tube. *C. heterophylla* (fig. 122) provides an example of the former; *C. maxima* (fig. 119), an example of an intermediate state; and *C. cornuta* (fig. 109), an example of the formation of a tube after the lateral fusion of the tertiary bracts. The secondary bracts are lacking.

The vascular systems of the different types of cymules in this genus do not differ appreciably, therefore a description of that of *C. cornuta* (fig. 118) will perhaps suffice.

Study of earlier developmental stages indicates many similarities to that of *Ostryopsis*, especially since the secondary and tertiary axes are so strikingly abbreviated. In the earlier stages of development the vascular supply to a given cymule departs from the vascular cylinder of the primary axis in the usual way, and the supply of the primary bract is independent of the rest of the vascular system of the cymule (fig. 110). The vascular supply to the rest of the cymule arises from the sides of the gap left by the primary bract bundle.[9]

These bundles form a short dictyostele representative of the

[8] *C. americana, C. avellana, C. californica, C. cornuta, C. colurna, C. davidiana, C. heterophylla* var. *sutchuensis, C. tibetica, C. vilmorinii, C. maxima.*

[9] This condition is obscured in the series shown in figs. 110–117 because of the great development of one of the florets at the expense of all the other structures in the ament, so that the proportions are tremendously modified.

secondary axis, which broadens laterally and separates into two arcs, one for each tertiary axis. But at this time there is no evidence of a vestigial vascular supply to the lost secondary bracts. Between the two arcs there remain the traces *y*, *y* and *z*, *z* which are the sole remnants of the secondary vascular axis. These traces ultimately migrate back toward the primary vascular axis and there take up a position in the gap left by the departure of the vascular tissue to the cymule. The two arcs of tissue representing the two tertiary axes tend to form dictyosteles, and at the same time a broad band of vascular tissue passes off to the abaxial tertiary bract. At a slightly higher level the traces to the adaxial tertiary bract pass off leaving behind a very short pedicellar cylinder of vascular tissue.

At the developmental stage shown in the reconstruction in figure 118 and in figures 110–177, the vascular supply to the other floret of the cymule is shown departing to the left in figure 110, while the vascular supply to the undeveloped distal portion of the ament is shown toward the top of the figure. The cylinder of tissue in figure 111 is the representative of the tertiary axis of the larger floret only in the cymule shown in figure 109. This dictyostele rapidly breaks up (fig. 112), sending off branches centrifugally to supply the tertiary bracts and also branches centripetally which represent the continuation of the tertiary vascular axis into the base of the floret. The system is much modified by anastomoses (*anas*) which enter into the ovary supply and will be considered in a discussion of the vascularization of the florets of the Betulaceae. With the increase in size of the cymule the bundles of the tertiary bract branch more profusely. A similar tendency has been noted in *Carpinus*, *Ostrya*, and *Ostryopsis*, where there is great development in size of the floret ovary in close proximity to the place of departure of the bract bundles from the very short axes of the cymule.

The dissection of the vascular system of the pistillate cymule in *Corylus* developmental stages appears to be associated with the extraordinary increase in the size of one of the two florets of the cymule in a notably telescoped inflorescence. Since the full influence of such factors as size on the internal morphology of the vascular system is by no means clear, although its significance is recognized,

there is no accepted basis for attributing definite cause-effect relationships to such coincidences as have been observed.

In general, however, the vascular system is essentially that of a cymule in which, surprisingly enough, the secondary bracts are absent, and in which there is excessive development in size of one of the florets of the cymule, resulting in disruption of the symmetry of the vascular system. The greatest similarity is with the vascular supply of *Ostryopsis* since there is in both the same abbreviation of the secondary and tertiary axes. This may be a simple case of parallelism, however, since the striking difference in the presence or absence of the secondary bract must be considered as well.

### Staminate cymules: morphology and anatomy

While there is much variation in the gross morphology of the pistillate ament, there is marked uniformity in that of the staminate ament, and it is here that the popular concept of the ament finds justification. The staminate aments are elongate, pendulous, and in many cases deceivingly simple in the morphology of their component cymules. In these cymules much variation from one group of species to the next is to be found, although all three florets are almost universally represented. This will be treated in some detail under the various genera, but basically the staminate cymules adhere to the interpretation accorded the pistillate cymules and the same principles of interpretation of the vascular system may be used in both cases.

From the anatomical point of view, the species studied possess in common the absence of secondary thickening in the xylem, both in the primary axis and in the axes of the higher orders. The evidence indicates that the vascular relationships of the leaf to the axillary branch in a system where concrescence plays a prominent rôle is applicable throughout. Absence of secondary thickening appears to be correlated with the time factor, since secondary xylem is also lacking at a corresponding period in the development of the pistillate aments. Owing to the fall of the staminate aments after the shedding of the pollen there is of course no further opportunity for secondary xylem to form.

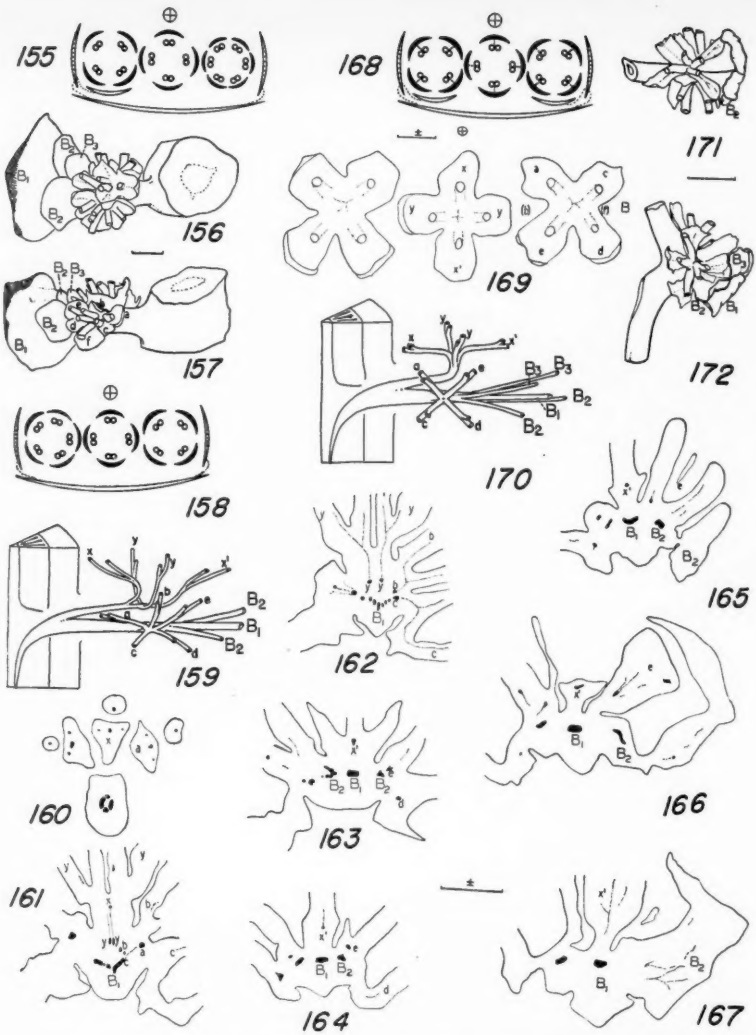
*Alnus, staminate cymules*

A study of a number of species[10] indicates that there is a great range of variation in the morphology and anatomy of the individual cymules of this genus.

The habit sketches of a cymule of *A. firma* var. *hirtella* (figs. 156, 157) are representative of the members of s. Gymnothyrsus. In addition to the primary bract, both secondary bracts are present but only one of the tertiary bracts occurs. Other cases have been found in which both of the abaxial tertiary bracts (the adaxial bracts are uniformly absent) are present, or more rarely both abaxial tertiary bracts may be absent.

The vascularization of a cymule of *A. crispa* var. *mollis* is shown in figure 159 and the following transverse sections (figs. 160–167). The vascular supply to a cymule originates much as it does in the pistillate material, a single trace deriving from the base of an elongate gap in the vascular cylinder of the primary axis. This trace grows larger as it passes outward into the base of the cymule, becomes cylindrical by the overarching of its margins (fig. 160), and then separates into (1) an upper portion which represents the continuation of the secondary axis and which ultimately supplies the secondary floret (*y* and *y*, fig. 161), and (2) a lower portion which continues for a time in a loose association with the other vascular tissue (figs 161, 162) but soon becomes independent (fig. 163) and ultimately supplies the primary bract (fig. 164 *et seq.*). From the vascular tissue flanking the primary bract bundle there differentiates the secondary bract bundle, which has associated with it the various traces which supply the tertiary florets and represent the much telescoped tertiary vascular axis. Where the tertiary abaxial bracts are present they derive their vascular supply from between the secondary and primary bundles, just as in the pistillate cymules.

[10] Section Alnobetula: *A. firma* var. *hirtella*, *A. crispa* var. *mollis*, *A. sinuata*.
Section Gymnothyrsus: *A. incana*, *A. jorullensis*, *A. hirsuta*, *A. hirsuta* var. *sibirica*, *A. tenuifolia*, *A. spaethii*, *A. rhombifolia*, *A. rubra*, *A. maritima*.
Section Clethropsis: *A. nitida*, *A. nepalensis*.
Section Cremastogyne: *A. cremastogyne*, *A. lanata*.




FIGS. 155–172.—Figs. 155–157, *Alnus firma* var. *hirtella*, staminate: fig. 155, floral diagram for two figures following; fig. 156, dorsal view of cymule*; fig. 157, lateral view. Figs. 158–167, *A. crispa* var. *mollis*, staminate: fig. 158, floral diagram of cymule shown in following figures; fig. 159, reconstruction of vascular system of cymule shown in following figures; fig. 160–167, successive transverse sections. Figs. 168–170, *A. incana*, staminate: fig. 168, floral diagram of cymule shown in following figure; fig. 169, the three florets removed and placed in one plane in normal orientation to one another; fig. 170, reconstruction of vascular system. Figs. 171, 172, *A. nitida*, staminate: dorsal and lateral views respectively.

* The anthers have been removed in practically all staminate cymules figured so that filaments only represent the stamens.

*A. firma* var. *hirtella* departs in certain respects from the preceding type of vascularization in that the origin of the vascular supply to the cymule is three-parted, as it is in the typical branch-bract association, the median bundle ultimately supplying the primary bract and the two bundles arising from the sides of the gap providing the vascular supply to the rest of the cymule. The bundles *z* and *z*, mentioned in connection with the anatomy of the pistillate cymules of *A. subcordata*, *Corylus* and *Ostryopsis*, appear in this species also and replace in their function the bundles *y* and *y*. The presence of *z* and *z* on either side of the primary bract bundle supplies a portion of the secondary axis vascular system lost in the other species of this section. Furthermore the trace *e* in this species arises from between the bundles to the secondary and tertiary bracts, which emphasizes the similarity between the staminate and pistillate vascular systems.

As shown in the reconstruction (fig. 170), the vascular system of a cymule of *A. incana*, representative of most of the species of s. Gymnothyrsus, is essentially like that of the species of the preceding section except that the tertiary bracts are regularly present. The secondary floret, as usual, is supplied by the traces *x* and *y*, *y* representing the secondary axis. The tertiary axis is composed of the bundles *a* and *c* arising from the ventral flank of the secondary bract trace, and by *e* and *d* arising from between the secondary bract trace and the tertiary bract trace. In *A. rhombifolia* and *A. jorullensis* the secondary axis is reduced to even greater simplicity by the presence of only the trace *x*, while the tertiary axis is represented only by the fusion bundle *e* plus *d* which arises from between the bundles supplying the secondary and tertiary bracts of the floret involved. *A. rubra* differs from the rest of the *A. incana* group in the formation of the secondary axis from the traces *x* and *y*, the last arising from the sides of the primary bract trace.

In only one case has a cymule been found in which the secondary floret was lacking, thus presenting a similarity to the regular pistillate condition. In the distal portion of the ament in *A. tenuifolia* one such cymule was found, which upon sectioning showed a condition like that of the three-flowered cymules. Here the traces *x*, *y*, and *y* after becoming differentiated become closely approxi-

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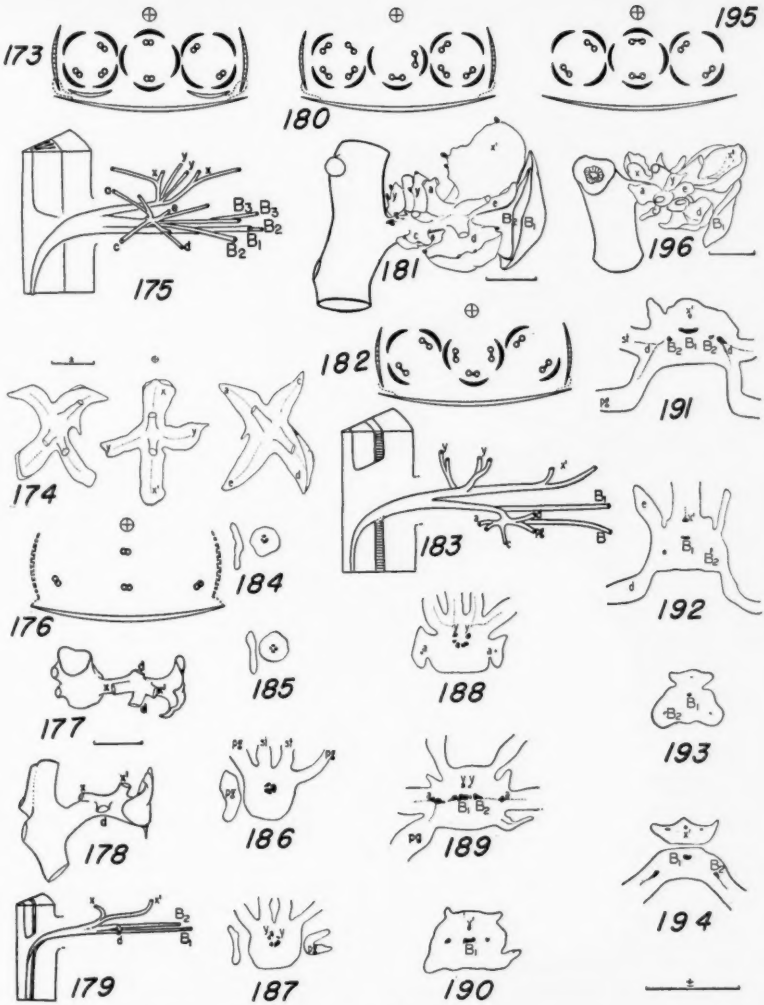
mated with the bundles to the tertiary bracts, much as do the homologous traces in the normal two-flowered pistillate material.

The cymules of the species composing s. Clethropsis are strikingly different from those of the preceding two sections in the marked shortness of the secondary and tertiary axes, bringing the florets very close to the primary axis of the ament (figs. 171, 172). In both species, bracts of the second and third order are present in addition to that of the first order, but the range of floret conditions covers that of the species of both the preceding sections. In the distal portion of the aments of *A. nepalensis*, the bracts of the third order and then those of the second order are absent from the cymules. There are corresponding reductions in the number of parts present in the individual florets.

The vascular system of the cymules of the species studied from this section is essentially like that of the preceding two sections, except that, as in the pistillate cymules of these species, there is early division of the bundles ultimately supplying the cymule, so that there is a well defined dictyostele in the short secondary axis.

Both species of s. Cremastogyne agree well in the morphology of their cymules. This is illustrated for *A. lanata* in figures 177 and 178. There is no external evidence of a condition similar to that just described for the cymules of the terminal portion of the ament of *A. nepalensis*. The vascular system is shown in figure 179. The supply to the cymule originates as a single small mass of xylem at the base of a gap.

A dictyostele forms by the overarching of this small bundle, while the traces *y* and *y*, and *x* depart in the usual way to form the vascular system of the secondary axis. The bundle supplying the primary bract differentiates opposite to *x* and supplies the primary bract. This bundle is flanked by two diminutive bundles which parallel it and doubtless represent the vestiges of the supply to lost secondary bracts since the trace *d* departs from the outer side of it. Trace *d* represents the sole remaining portion of the tertiary vascular axis.




FIGS. 173–196.—Figs. 173–175, *Alnus rhombifolia*, staminate: fig. 173, floral diagram for following habit sketch; fig. 174, three florets of cymule dissected off and shown in relative positions; fig. 175, reconstruction of vascular system of cymule similar to that shown in preceding figure. Figs. 176–179, *A. lanata*, staminate: fig. 176, floral diagram of cymule; figs. 177, 178, dorsal and lateral views respectively; fig. 179, reconstruction of vascular system of cymule shown in preceding two figures. Figs. 180, 181, *Betula lenta*, staminate: floral diagram and lateral view of cymule respectively. Figs. 182–194, *B. lutea*, staminate: fig. 182, floral diagram; fig. 183, reconstruction of vascular system of cymule (very similar to that of *B. lenta*); figs. 184–194, successive transverse sections upon which preceding reconstruction is based. Figs. 195, 196, *B. alnoides* var. *pyrifolia*, staminate: floral diagram and lateral view respectively.

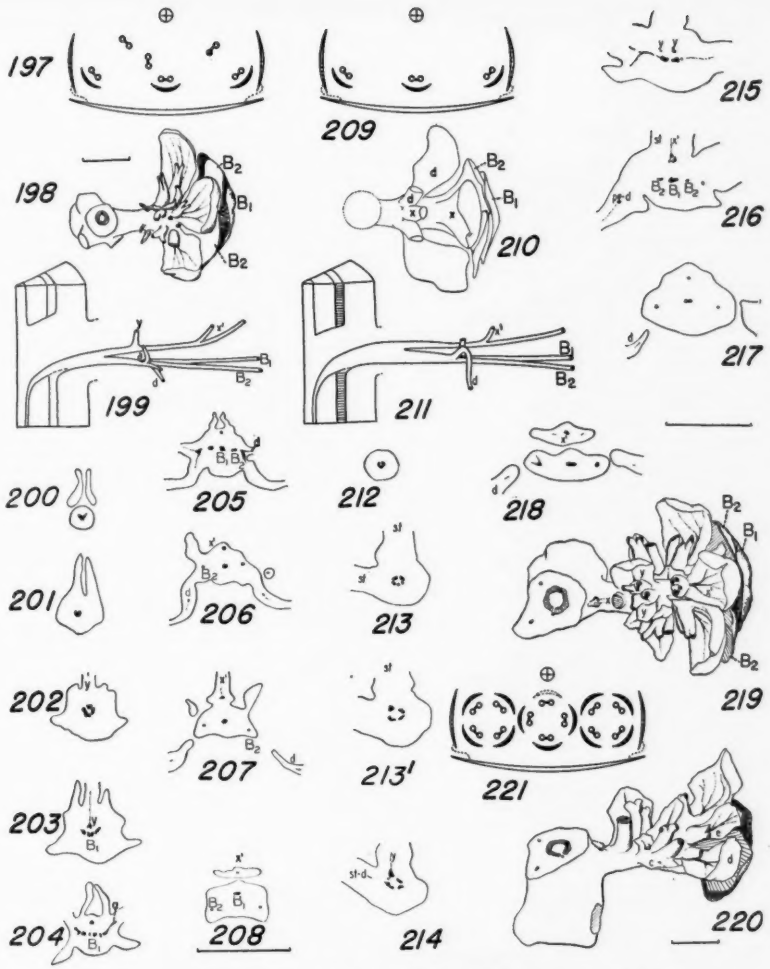
*Betula, staminate cymules*

A general survey of the cymules of the species studied[11] in this genus shows the striking similarity between the cymules of the birches and those of some of the alders. The condition which is typical in *Betula* has been foreshadowed and in some cases surpassed in *Alnus*, namely, the absence of the tertiary bracts. As in the s. Cremastogyne of *Alnus*, even the secondary bracts are absent in the ss. Acuminatae of *Betula*.

A consideration of the anatomy of the cymules of the genus may well be based on that of *B. lutea* (fig. 183). The vascular system to a cymule originates as a single strand from the base of the gap. This strand becomes augmented as it approaches the cortex, there becomes lunate, and finally forms a loose siphonostele out in the basal portion of the cymule (fig. 184). This small vascular cylinder is that of the secondary axis of the cymule. From the upper portion of this loosely organized cylinder there depart the two traces *y* and *y* representing the continuation of the secondary vascular system beyond the differentiation of the tertiary vascular axis (figs. 186–188). It is from these traces that the supply to the secondary floret is derived. The remaining vascular tissue of the cymule consists of the primary bract bundle, which because of concrescence is thus long associated with the vascular system of the cymule, and two more or less well defined arcs of tissue flanking the secondary bract bundle on either side. These arcs represent the two tertiary vascular axes from which the vascular tissue of the tertiary florets and secondary bracts is derived in much the same way as in *Alnus*. The major

[11] Section Eubetula:

| | |
|---|---|
| Subsection Costatae: | *B. lenta, B. lutea, B. grossa, B. nigra.* |
| Subsection Albae: | *B. coerulea-grandis, B. japonica, B. papyrifera* var. *carpatica, B. papyrifera* var. *occidentalis, B. pendula, B. populifolia.* |
| Subsection Nanae: | *B. glandulosa, B. michauxii, B. pumila.* |
| Section Betulaster: | |
| Subsection Acuminatae: | *B. alnoides* var. *pyrifolia, B. luminifera, B. maximowicziana* (material of this species is from a tree in the Arnold Arboretum and is almost surely hybrid, the female parent being *B. maximowicziana*, the male parent unknown). |




FIGS. 197–221.—Figs. 197–208, *Betula pendula*, staminate: fig. 197, floral diagram; fig. 198, dorsal view of cymule; fig. 199, lateral view of reconstruction of vascular system; figs. 200–208, successive transverse sections through cymule similar to that shown in fig. 198. Figs. 209–218, *B. pumila*, staminate: fig. 209, floral diagram; fig. 210, dorsal view of cymule; fig. 211, reconstruction of vascular system similar to that shown in preceding figure; figs. 212–218, successive transverse sections. Figs. 219–221, *B. maximowicziana*, staminate cymule: dorsal and lateral views and floral diagram respectively.

difference is that the tertiary vascular system in *B. lutea* is represented by only a single compound bundle derived from the upper shoulder of the secondary bract bundle.

There are few departures from this general plan. Only in *B. maximowicziana* is the bundle *x* found to be present as it occurs in the staminate cymules of *Alnus*. In the species of ss. Acuminatae which have lost the secondary bracts there is not even a vestigial secondary bract trace, but otherwise the vascular system resembles that described for *B. lutea*.

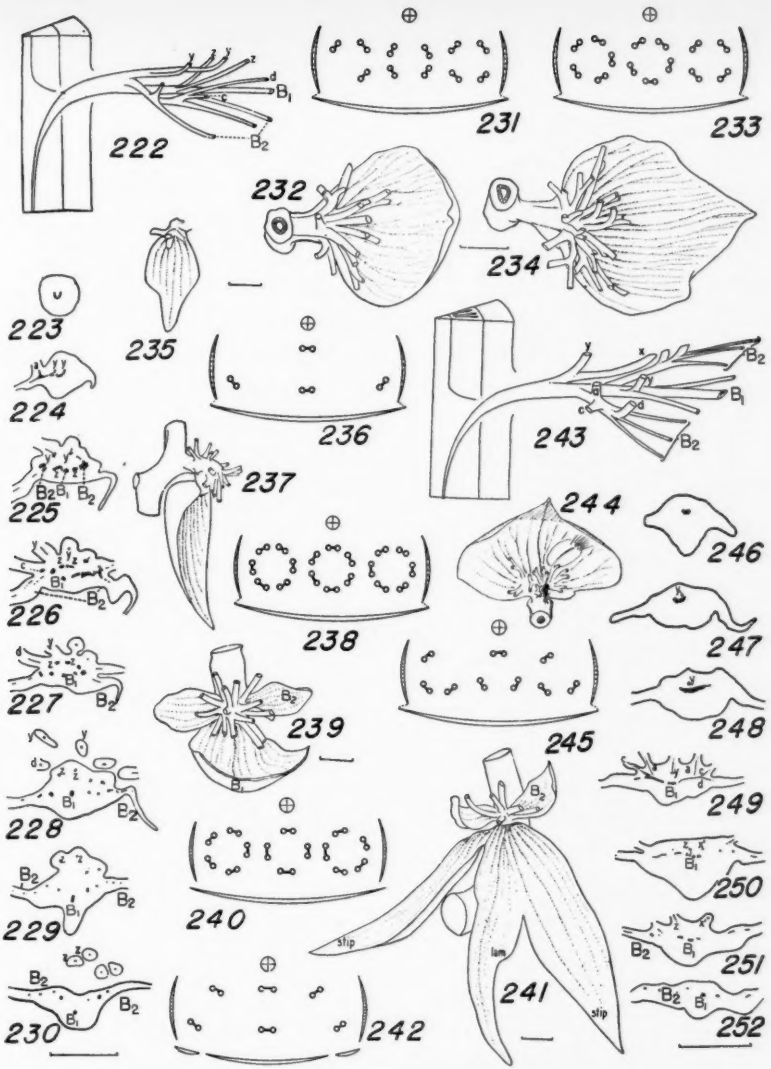
Thus it is clear that a notable uniformity of vascular plan characterizes the genus as a whole, with the species of ss. Acuminatae standing apart because of the absence of the secondary bracts and the corresponding bract bundles, a condition parallel to that occurring in the s. Cremastogyne in *Alnus*.

### *Carpinus, staminate cymules*

Of the species studied,[12] the most significant in view of what has gone before is *C. japonica*, since there one finds in the cymules at the base of the aments the free secondary bracts (fig. 239). That these are indeed bracts and not stipules is shown by their presence in addition to stipules (fig. 241) in the cymules from a few nodes farther down in the transitional region between twig and ament. At higher levels (figs. 237, 235) these secondary bracts are no longer distinguishable externally. In the other species of the genus the cymules are similar to the examples illustrated (figs. 232, 234). Confusion in the interpretation of the morphology of the cymule in this and the following genera has long existed because of the absence of tepals in the normal florets. It is clear from the vascular systems, however, that there are actually three florets composing each of the cymules. As indicated in the habit sketches, the bracts are richly supplied with small veins. While these are referable to three main systems corresponding to the primary and the two secondary bracts, this is obscured by their branching at a very early stage, recognizable in most cases only by sectioning.

[12] Section Eucarpinus: *C. betulus, C. caroliniana, C. laxiflora, C. orientalis, C. turczaninowi.*

Section Distegocarpus: *C. japonica, C. cordata.*




FIGS. 222–252.—Figs. 222–230, *Carpinus betulus*, staminate; fig. 222, reconstruction of portion toward the observer of vascular system, based on figs. 223–235; figs. 223–230, successive transverse sections through cymule similar to that shown for *C. laxiflora*. Figs. 231, 232, *C. laxiflora*, staminate: floral diagram and dorsal view of cymule respectively. Figs. 233, 234, *C. caroliniana*, staminate cymule: floral diagram and dorsal view respectively. Figs. 235–242, *C. japonica*, staminate cymules: figs. 235, 236, dorsal view and floral diagram respectively from terminal portion of ament; figs. 237, 238, lateral view and floral diagram from central portion of ament; figs. 239, 240, dorsal view and floral diagram respectively from near base of ament (note free secondary bracts); figs. 241, 242, dorsal view and floral diagram respectively in transition region between ament and twig (note reduced lamina, *lam;* persistent stipules, *stip;* and free secondary bracts). Figs. 243–252, *Ostrya virginiana*, staminate cymule: fig. 243, reconstruction of vascular system shown in section in following figures; figs. 244, 245, dorsal view and floral diagram; figs. 246–252, successive transverse sections similar to that shown in fig. 244.

The vascular system of *C. betulus* (fig. 222) characterizes that of the other species of the genus. The origin of the vascular system, like that of *Betula*, is in a single small bundle which arises at the base of a gap. This trace becomes lunate (fig. 223) but not siphonostelic, and from the upper margins gives off the two bundles *y* and *y* (fig. 224) of the secondary axis. The trace to the primary bract arises from the median lower portion of the vascular system (fig. 225), and on either side there arise the traces *z* and *z*, which constitute the remainder of the vascular system belonging to the secondary axis (figs. 225–228). Laterally there departs the vascular tissue composing the tertiary axis and that of the secondary bract associated with it (fig. 226 *et seq.*). So similar is this behavior of the vascular system to that obtaining where there are obviously three florets present that there can be no doubt that here there are also three florets bearing the same relationship to the cymule as do the florets in the other genera. This is emphasized by a parallel case observed in the staminate cymules of *Alnus*, s. Cremastogyne, and in the pistillate cymules of *Betula*, ss. Clethropsis. Furthermore the vascular system of the cymules with free secondary bracts in *C. japonica* (figs. 239, 241) differs in vascularization from the cymules of the other species in that the secondary bract traces pass to organs which are free rather than fused laterally to the primary bract. That tertiary florets occur in the axils of these secondary bracts is clear because of the close relationship between their respective vascular systems.

*Ostrya, staminate cymules*

The species studied are those noted under the description of the pistillate cymules (see footnote 7).

The external morphology, together with the distribution of vascular tissue of the cymules in this genus, is so like that in the majority of species described for *Carpinus* that no further description is necessary. A reconstruction of the vascular system and serial transverse sections are provided in figures 243, 246–252.

*Corylus, staminate cymules*

The species studied are those mentioned in the description of the pistillate cymules (see footnote 8). This genus presents an interesting and unique morphology. The secondary bracts, which are

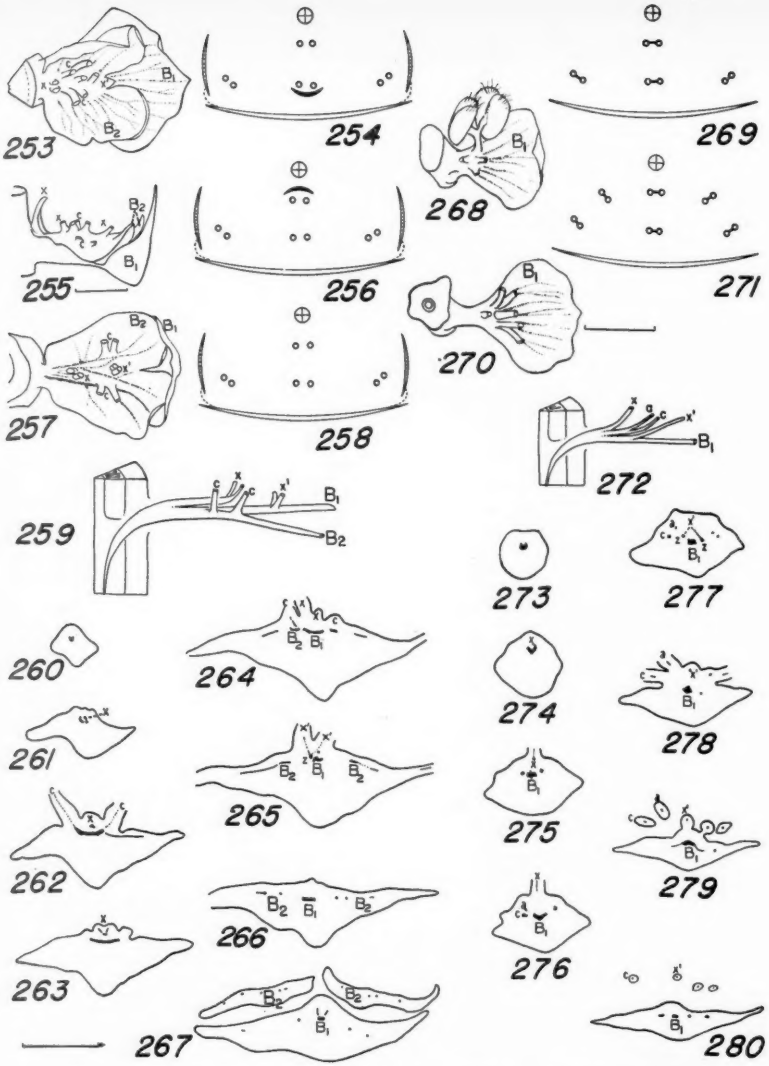
regularly present, are well developed and dorsiventrally fused to the primary bract. The individual florets are difficult to distinguish because they lack tepals and because the secondary floret is dimerous while those of tertiary origin are monomerous. The vascularization of the cymules, however, definitely establishes the fact that there are three florets involved. The vascular system originates as a single bundle from the base of the gap in the primary vascular cylinder, proceeds out into the base of the cymule, and there forms a diminutive siphonostele (figs. 260, 261). From the upper portion of this stele there departs the single bundle *x*, which is all that remains of the pedicellar portion of the secondary vascular axis (figs. 261, 262), while the remainder of the vascular tissue flattens out dorsiventrally (figs. 262, 263). From each side of this flattened vascular mass a secondary bract bundle separates. At the same time the trace *c*, representative of the tertiary axis, departs laterally from the secondary bract bundle (figs. 264, 265). From the space between the secondary and primary bract bundles originates the trace *z*, homologous with the same trace in the pistillate material of this genus, which completes the secondary vascular system. Thereafter the primary and secondary bract traces proceed unmodified except for branching into their respective bracts (fig. 265 *et seq.*).

The presence of secondary bracts and the intimate relation between their vascular supply and that of some of the floral members indicate that these members are to be considered the tertiary florets, which strengthens the conclusion already reached that the staminate cymule of *Corylus* is three-flowered. Furthermore a striking similarity exists between the vascular system of the cymules here and those in the preceding two genera, so that the evidence concerning the interpretation of the cymule as three-flowered is mutually corroborative.

*Ostryopsis, staminate cymules*

The same species were studied as in the pistillate material.

Morphologically (figs. 268, 270) and anatomically (fig. 272) the cymules are very simple in this genus. Essentially the same condition exists as in the cymules from the terminal portion of the aments in *Carpinus japonica* previously described. No secondary bracts nor traces to secondary bracts are present, and the members




FIGS. 253–280.—Figs. 253, 254, *Corylus americana*, staminate cymule from near proximal portion of ament, dorsal view and floral diagram respectively. Figs. 255, 256, *C. maxima*, staminate cymule from proximal portion of ament, lateral view and floral diagram respectively. Figs. 257, 258, *C. heterophylla* var. *sutchuensis*, staminate cymule showing usual condition in the genus, including preceding two species. Figs. 259–267, *C. cornuta*, staminate cymule: fig. 259, reconstruction of vascular system; figs. 260–267, successive transverse sections. Figs. 268–280, *Ostryopsis davidiana*, staminate cymules: figs. 268, 269, dorsal view and floral diagram respectively with but a single stamen in each of tertiary florets; figs. 270, 271, dorsal view and floral diagram with two stamens in each of tertiary florets; fig. 272, reconstruction of vascular system of type shown in fig. 270; figs. 273–280, successive transverse sections.

of the central floret are supplied by the traces *x* (figs. 274, 275) and *z* (fig. 277) while those to the tertiary florets are supplied by the traces *a* and *c* (fig. 276). The course and origin of the vascular system in the cymules of this genus are simplified versions of those described for the preceding three genera. This genus apparently reaches the ultimate in simplification of the staminate cymule by reduction for this family.

### Summary of cymule morphology and anatomy

In the interpretation of the inflorescence anatomy of the Betulaceae it is necessary to postulate only that the inflorescence is essentially a branch system. In utilizing this hypothesis the simple facts of nodal anatomy as set forth in the introduction constitute the working premises. In essence these are as follows: (1) that in a unilacunar condition the vascular supply to the bract arises from the base of the gap, while the vascular system of the axillary branch is derived from the mutual overarching of two bundles, derived from opposite sides of the gap (fig. 1); (2) that under conditions in which the internodes are short and the gaps do not close, the vascular cylinders become dictyostelic (fig. 2); (3) that if concrescence of the bracts is superimposed upon this, the bract traces appear to depart from the vascular cylinder of the axis of the next higher order (fig. 3); (4) that when dorsiventral and lateral pressures are introduced the courses of the bundles to the bracts are modified as the positions of the bracts are modified although their origins are but little affected (fig. 4); and (5) that it would seem a logical corollary of (4) that where pressure is greatest the organs are most likely to be suppressed and their vascular system ultimately lost as well.

In the Betulaceae, in the pistillate cymules of *Alnus* when these cymules are three-flowered (fig. 17)—as in proliferated material—the nearest approach (fig. 19) is made to a simple type of vascular system, such as that shown in figure 3. In the absence of the secondary floret (fig. 21) the traces *x* (fig. 20) and *y* (fig. 65) still exist as vestigial traces representing the secondary vascular system; and as is often the case with vestigial vascular bundles, they tend to fuse with certain of the adjacent vascular bundles. The reverse of this condition is found in the staminate cymules of *Alnus*, which are

regularly three-flowered (fig. 168) and which have the complement of vascular tissue (fig. 175) associated with the pistillate three-flowered cymules. In the one case found in which the secondary floret of the staminate cymule was absent, however, the vestigial bundles of the secondary vascular axis behaved very like that of the two-flowered pistillate cymules.

Associated with the tendency toward reduction, the vascular system shows corresponding modifications, namely, that with the disappearance of a bract its bundle also tends soon to disappear (phylogenetically speaking). Thus the loss of the secondary bracts (fig. 176) in the staminate cymule of the species of s. Clethropsis is correlated with a strong reduction in the size of the bundle to this organ (fig. 179).

In *Betula*, the same general plan (figs. 197, 77) obtains in both the staminate and pistillate cymules, with the tertiary bracts lost completely and the secondary bracts in the process of disappearance in some of the species (fig. 88). Associated with this is a decided reduction in the entire extent of the vascular system (figs. 67, 183), as well as in the general habit of growth of the aments which are not so long-lived as they are in *Alnus*. There is a strong similarity between the two genera in their external morphology and in the distribution of their vascular tissues, indicating a common origin.

In *Carpinus* many features are found which suggest a close relationship to some less modified ancestral type, especially in the freedom of the several bracts from one another in the pistillate cymules of some of the species. There are other stages (figs. 138, 152) showing tendencies toward fusion of the tertiary bracts laterally to those of secondary nature, but in their vascular organization such cases are closely similar to those in which the bracts are free. Corresponding tendencies (figs. 238, 240) were noted in the staminate cymules, although no evidence was found of the presence of tertiary bracts. In at least one species, however, secondary bracts were found to be free from the primary bract in the basal portion of the ament (fig. 240), thus explaining the presence of the vascular supply to the secondary bract in other forms in this genus even when there appears to be no external evidence of such a bract. This may be attributed to the lateral fusion of these organs with the primary

bracts, just as the tertiary bracts fuse laterally with the secondary bracts in the pistillate cymules.

The absence of the tepals, which provide the most obvious means of differentiating these closely crowded florets aside from the vascular system, has misled those who have studied only the external morphology of the staminate cymules (fig. 232) of *Carpinus*. The vascular system (fig. 222), however, is so similar to that of other members of the family (fig. 175) where there is indubitable evidence of the presence of the three florets in the cymule, that there can be little hesitation in according a similar interpretation to these.

*Ostrya* so closely follows the lead of *Carpinus* that it needs but little comment. The pistillate cymules have proceeded but a step beyond those of *Carpinus* in that the tertiary bracts are fused laterally to each other as well as to the secondary bracts, so that each of the two florets of the cymule is surrounded by an involucre (fig. 136). Anatomically (fig. 124) this condition is close to that of *Carpinus*. The staminate cymules also are like those of *Carpinus* morphologically (fig. 244) and anatomically (fig. 243), and admit of a similar interpretation (fig. 245).

*Ostryopsis* is of interest because, although the pistillate cymules (fig. 99) are intermediate between *Carpinus* and *Ostrya* in the behavior of their bracts, in that the tertiary bracts are fused laterally to the secondary bract, the bracts are all of the same length and are furled around the individual florets, thus simulating the condition found in *Ostrya*. But the secondary and tertiary inflorescence axes are so shortened that the individual florets are practically sessile on the primary axis of the ament. It is only the vascular system which indicates that the various organs bear the same relationship to each other as they do in *Carpinus* and *Ostrya*. In this reduction of the tertiary axes the genus bears a closer similarity to *Corylus* than it does to the preceding two genera. Also in the morphology (figs. 268, 270) of the staminate cymules it bears a similarity to *Corylus*, although this will become more obvious after the anatomy and morphology of the individual florets have been described. There is, moreover, no external nor internal (fig. 272) evidence in the staminate cymules of the existence of tertiary or secondary bracts, in-

dicating an extreme of reduction of these organs similar to that found only in the pistillate cymule of *Betula michauxii* (fig. 85).

*Corylus* stands apart as an unusual genus although it bears some likeness to the other genera already mentioned. The individual pistillate cymules (fig. 122), of which there are but few in an ament, lack the secondary florets as do the pistillate cymules of the preceding three genera. In the uncultivated species, one of these tertiary florets ordinarily gains the ascendency in the course of development. An additional peculiarity is the absence of the bracts of the second order, a condition which results in the formation of an involucre composed entirely of tertiary bracts. Thus the involucre in those species in which the bracts have "fused" laterally (fig. 109) instead of remaining free (fig. 122) is by no means homologous with that of *Ostrya* (fig. 136) which it resembles superficially. Not even the vascular system of the secondary bract is present in *Corylus* (fig. 118) as it is in the involucre of *Ostrya*. The staminate cymules on the other hand possess the secondary bracts which are fused dorsiventrally to the primary bracts but lack the tertiary bracts. In addition all three florets of the cymule are represented (fig. 258).

In all these cases it is found that the mode of origin of the vascular system to the cymules, pistillate or staminate, is remarkably uniform, in many cases being of the most diagrammatic nature. While the primary bundle trace often remains in close apposition to the rest of the vascular tissue of the cymule, its identity and distinctness is never to be mistaken and readily lends itself to interpretation as the result of concrescence of that organ to the remainder of the cymule. The vascular tissue usually forms a definite siphonostele, or at least a dictyostele representative of the secondary inflorescence axis. From this siphonostele there depart laterally the bundles to the secondary bracts. Concrescence again plays a part here, so that the secondary bract trace often maintains a close relationship with the tertiary vascular system until after the departure of the tertiary vascular system from the secondary vascular axis. So intimate is the union of the vascular tissue of the tertiary axis with that of the secondary bract, that it often appears to be derived laterally from the secondary bract trace, a condition which nevertheless maintains the basic relationship between the vascular system of a bract and

its axillary branch. When tertiary bracts are present, they depart just as might be expected in a telescoped cymule, being closely associated with the vascular tissue of the tertiary axis as illustrated in the hypothetical case in figure 4.

Thus it is evident that the vascular system of the cymule, pistillate and staminate, supports the accepted interpretations of morphology in more obvious forms, and makes clear the basic morphology in more obscure forms.

## Floret morphology and anatomy

Floral morphologists have long endeavored to explain the nature of the florets in the Betulaceae. In the one group, the Coryleae, the ovary has been accepted as inferior because there is tangible evidence of the presence of a perigon. But in the Betuleae, while the question has been raised of the apparent absence of perigon in a group of genera obviously close to the Coryleae, the answer has never been attempted in more than one or two isolated cases, nor has a study been made of the vascularization of the florets.

A second problem is that of the diverse orientation of the ovaries in the secondary pistillate florets, which have ordinarily been described as median to the secondary bracts in *Alnus*, *Betula*, and *Corylus*, and transverse in *Ostrya* and *Carpinus*.

The problem of the basic morphology of the florets of the Betulaceae may best be attacked by first considering the morphology and anatomy of the staminate florets.

### Staminate florets: morphology and anatomy

*Alnus, staminate florets* (for species studied see footnote 10).

The individual florets (of which three are present in each cymule) in this genus regularly have a well developed perigon, with the exception of the species of s. Cremastogyne. The number of tepals varies from six to one, depending on the species. The florets of the species of s. Alnobetula are hexamerous to tetramerous, the range being shown in the single cymule of *A. firma* var. *hirtella* in figures 156 and 157 (*cf.* floral diagram, fig. 155). The hexamerous condition is rather uncommon, but when present (fig. 157) the tepals are in two whorls of three members each, as are also the stamens. No

unusual condition exists in the vascular supply to these floral components, other than the fusion of the stamen traces to the tepal traces somewhat beyond the point of origin of the tepal traces. Beyond the departure of the stamen trace, the tepal trace becomes tripartite (fig. 160). In s. Gymnothyrsus, the individual florets are commonly tetramerous as in *A. incana* (fig. 169); but in some species, such as *A. rhombifolia* (fig. 174), there is a strong tendency toward trimery or dimery of the stamens, and also in some cases toward the reduction of the number of perigon segments. In *A. incana* the fusion of the stamens to the tepals is so complete that it involves the entire filament, but otherwise the vascular system of the floret (fig. 170) is normal. In the vascular system of a floret in which the stamens confronting certain of the tepals may be lacking, the vascular system of such tepals is generally developed as strongly as though the stamen associated with it were present. In the reduction of the components of the secondary floret, it is the lateral stamens which are first lost.

The individual florets of s. Clethropsis show many similarities to those of the two sections just mentioned, in that the whole range from pentamery to dimery is covered. They differ in the smaller absolute magnitude of the mature florets, and in the greatly exaggerated length of the filaments. In figures 171 and 172 are shown two views of a cymule of *A. nitida* with a tetramerous secondary floret and a pentamerous tertiary floret. In *A. nepalensis* the tendency toward trimery and dimery is also strongly expressed. The vascular supply of each of these florets, as in other members of the family, is derived from either side of the gap left by the departure of the subtending secondary bract and therefore represents the tertiary axis. It is significant that the florets at the distal end of the ament in *A. nepalensis* often lack tepals, so that the florets are represented by their stamens alone, which are reduced in number to but one or two each.

In s. Cremastogyne, both species have the same type of floral morphology, which is essentially like that described for the terminal portions of the ament in *A. nepalensis*. The tepals are completely absent (*A. lanata*, figs. 176–179) and but one stamen, *d*, remains to represent each of the tertiary florets while the secondary floret

is represented by two stamens, $x$ and $x'$. That three florets are present is indicated, not only by homology with the reduced cymules to be found in *A. nepalensis*, but also because each floret has its individual vascular supply which arises similarly to that in species where the florets are readily recognizable as distinct.

In this genus the whole gamut is run from hexamerous to monomerous florets, as well as from the presence of a complete perigon to its complete absence. Where a perigon is present, the filaments of the stamens are fused with the tepals for an appreciable distance, although this is masked in s. Clethropsis by the extreme length of the free portion of the filaments.

*Betula, staminate florets* (for species studied see footnote 11)

While radial symmetry predominates in the individual florets of the preceding genus, in the florets of the various species of *Betula* there is characteristically a tendency toward zygomorphism and toward the presence of divided anthers. The zygomorphism is correlated with the shortening of the secondary axis of the cymule, which crowds the individual florets against the primary axis of the ament. Associated with this pressure there is suppression of the floral parts toward the primary axis. This is least evident in available material of *B. maximowicziana* (figs. 219, 220). The secondary floret often has the tetramerous plan completely carried out in both the perigon and the androecium, but in the example shown (fig. 219) the adaxial perigon segment is represented only by a gland-tipped protuberance. These protuberances are of considerable importance in the floret morphology of the family as a whole, since the margins of the tepals are regularly studded with these glands. In general these glands have been omitted from the habit sketches because they so complicate them, but an idea of their mode of distribution on the perigon segments may be gained from figure 181. The glands persist in their association with the tepal in all stages of its reduction, and since these glands are of a peculiar elongated type, differing from the flat lenticular ones commonly occurring on the surfaces of the bracts of *Alnus* and *Betula*, their presence in the absence of a perigon segment is considered to be indicative of the former presence of a tepal. The tertiary florets (fig. 220) of *B. maximowicziana* are

also often tetramerous but are more zygomorphic than are the secondary florets. Trimery also occurs commonly in the florets of this species, and is achieved by the loss of the adaxial stamen of the secondary floret or one of the diagonal stamens in the tertiary florets.

Trimery occurs commonly in the florets of species in ss. Costatae, although an occasional tetramerous floret is present as in *B. lenta* (fig. 181). Dimery becomes the rule in *B. nigra* of this section.

In the ss. Albae, while trimery sometimes occurs, dimery of the individual florets is the rule, and there is a strong tendency toward the reduction of the number of tepals to one in each floret, with the other tepals represented by the stalked glands previously referred to, as shown in *B. pendula* (fig. 198).

In the ss. Nanae trimery is very rare, being found only occasionally in cymules from the base of the ament in *B. glandulosa*. Dimery is fairly common in the secondary florets, while the tertiary florets are generally monomerous; but the secondary floret may also be monomerous (*B. pumila*, fig. 210).

*B. alnoides* var. *pyrifolia* and *B. luminifera* of ss. Acuminatae differ from the other species of *Betula* thus far mentioned in the reduction of the lateral members, *y* and *y*, of the secondary floret, and by the persistence of perigon segments in the absence of the stamens associated with them, a situation rather reminiscent of some species of s. Gymnothyrsus (fig. 174) of *Alnus* than of the other species of *Betula*.

Throughout the genus the vascular system supplying the florets is very simple. Oddly enough the more reduced tepals often lack a vascular system, although they may still be recognizably foliose, such as tepal *c* in figure 196. Even if supplied with vascular tissue, this may be entirely unconnected with the main vascular system of the cymule. There may well be a direct correlation here between extent of vascularization and absolute size of the organ.

The origin of the vascular supply to the members of the florets composing the cymule has been described under the cymule anatomy. Whether the florets are tetramerous or monomerous, the origin of their vascular system is basically the same. The chief difference is in the number of individual traces, which depends on the number of parts the floret might have. Well developed tepals

have a three-parted vascular supply after the departure of the stamen bundle in *Alnus*.

*Carpinus, staminate florets* (see footnote 12)

The bewildering array of stamens and the absence of tepals in the cymules of this genus and in *Ostrya* have long been the occasion of a misinterpretation of the true morphology of the cymule. This confusion is increased by the division of the stamens extending far down into the filament proper. Upon removal of the anthers, it may be seen that the filaments tend to be arranged in three groups corresponding to the secondary and tertiary florets. As pointed out in the description of the vascular system of the staminate cymules in this group, the vascular supply to the stamens is homologous with that of other cymules in the family where three florets are obviously present. The individual florets are often hexamerous (*C. japonica*, fig. 237) in the species of s. Distegocarpus (sometimes up to 8-merous), and as either end of the ament is approached may become reduced to dimerous (*C. japonica*, fig. 242) or even monomerous (*C. japonica*, fig. 235). The species of the other section have their florets less often hexamerous, more commonly pent- or tetramerous (figs. 232, 234), and even trimerous.

*Ostrya, staminate florets* (see footnote 7)

Morphologically the florets of this genus are identical for all practical purposes with those of s. Eucarpinus of *Carpinus* with a tendency toward a smaller number of stamens per floret.

*Ostryopsis, staminate florets*

The regular thing observed in the two species of this genus is dimery of the secondary and tertiary florets (fig. 270), or dimery of the secondary and monomery of the tertiary florets (fig. 268). Otherwise the general condition is the same as that in *Carpinus* and *Ostrya*.

*Corylus, staminate florets* (see footnote 8)

As in *Ostryopsis*, the florets are either monomerous or dimerous. Tepals are also lacking. The stamens are completely divided to the base. Sometimes, in the proximal portion of the aments, the stamen

halves of the tertiary florets may be rather widely separated from each other (fig. 255); ordinarily, however, they are closely associated (figs. 253, 257).

Occasionally small foliose structures are found in the position of tepal $x'$ (fig. 253) or of $x$ (fig. 257) which have diminutive vascular supplies. This indicates that these may be tepals. In teratological specimens, to be discussed in the next paper of this series, the complete perigon complement of the secondary floret may occur, which further suggests that these are vestigial perigon segments in the normal material.

As pointed out in the discussion of the vascular system of the cymule, the origin of the vascular supply to the monomerous or dimerous florets in this genus is such as to establish their homology with other less reduced ones.

## Pistillate florets: morphology and anatomy

A striking diversity of external form characterizes the pistillate florets of the Betulaceae. In the genera of the Coryleae an easily recognizable perigon is present throughout, clearly confirming the fact that the ovary in this subfamily is inferior. But in the other subfamily, the Betuleae, although evidently closely related, no evidence of a perigon has been reported except by Wolpert (**36**).

### *Ostrya, pistillate florets*

The species studied in this genus (see footnote 7) possess the same general type of floral morphology as that shown for *O. virginiana* (fig. 135). The ovary is inferior owing to its fusion with a tetramerous perigon (*pg*, figs. 135, 136) which in the older stages is extended in a tubular form slightly beyond the body of the ovary. The vascular supply of the floret is derived from the dictyostele forming the tertiary vascular axis (*ped*, fig. 130). From this dictyostele a number of anastomosing branches (*anas*, fig. 131) progress radially inward to form the compound ventral bundle of the bicarpellary ovary, while the remaining portion of the dictyostele progresses upward into the perigon, forming the perigon supply (*pg*, figs. 131, 132). It is from members of this perigon supply that branches are derived to form the two dorsals of the ovary (*dr*, fig. 132). The dorsals arise

in the same plane as did the tertiary bract bundles, an aid in considering the orientation of the ovary. These dorsals continue upward throughout the length of the ovary and into the styles without further branching. The perigon supply passes with some branching into the perigon tube, while the ventral bundle divides laterally into two branches, each supplying an ovule (fig. 134). The division occurs in the septum separating the two loculi of the ovary. It is only after the ovule supply has departed that the septum disappears, permitting the formation of a fusion loculus. This loculus is continuous with the rather conspicuous interstylar canal and is a characteristic feature of the Betulaceae. Its presence is apparently correlated with the complete disappearance of the compound ventral bundle beyond the region of insertion of the ovules, so that there is no vascular tissue to support the loose parenchymatous tissue of the septum. The number of bundles supplying the individual tepal is variable, even within the same species or within the same cymule, but basically there seem to be three for each tepal as in the staminate florets of *Alnus*. With the close association of the tepals due to their lateral fusion, occasional adjacent perigon bundles have probably fused to form a compound trace,—a well known phenomenon in floral anatomy.

*Carpinus, pistillate florets* (see footnote 6)

These florets are very similar to those described for *Ostrya*, except that the perigon does not extend beyond the ovary. The tepals are generally represented simply as small teeth, as in *C. japonica* (fig. 139); although sometimes as in *C. caroliniana* (fig. 153) the individual teeth are three-lobed, lending support to the idea already expressed that the vascular supply to the tepals in this subfamily is basically tripartite. The vascularization of the florets is practically identical with that in *Ostrya*. The septum in the ovary disappears at the level of the division of the compound ventral bundle, which occurs slightly below the insertion of the ovules (fig. 150), giving the ovules the appearance of being parietal. This is, however, a secondary phenomenon. It is significant that sometimes it is the ovules of directly opposite margins of the two carpels that receive the vascular supply and sometimes it is the diagonally

opposite margins that are involved (fig. 150). It is rarely that all four ovules, one for each margin of each of the two carpels, develop. There does not seem to be any particular rule about which of the ovules develops in any given case. The supply to the ovules in these two genera is tripartite, with further branches arising from the two laterals in the base of the ovule. The dorsal bundles arise in the plane of the tertiary bract bundles, indicating that the same basic orientation exists in this genus as in *Ostrya*.

*Ostryopsis, pistillate florets*

In general the florets in this genus show a strong similarity to those in the preceding two genera, but the perigon tube is somewhat longer than its homologue in *Ostrya* and does not have such well defined teeth, but is nevertheless obviously tetramerous (*pg*, fig. 99).

The vascular supply of the florets is complicated by the shortness of the tertiary axis, so that the floral bundles depart shortly after the departure of the tertiary bract bundles (fig. 106). Nevertheless essentially the same type of floral supply exists here as in *Carpinus* and *Ostrya*. The tepal bundles differentiate immediately after the departure of the tertiary bract bundles; the ventral bundle forms by anastomoses from among the perigon bundles; and the dorsals arise as branches of the perigon bundles. The compound ventral bundle divides in the septum and supplies the two ovules, after which the septum breaks down, permitting the formation of a fusion loculus.

The dorsals arise in the plane of the abaxial tertiary bract bundle and that of the secondary bract bundle, so that the ovary is diagonal to the secondary bract.

*Corylus, pistillate florets* (see footnote 8)

The perigon of the florets in all the species studied in this genus is so reduced that it is recognizable only as an irregularly sinuate fringe (*pg*, figs. 109, 119, 122). It is difficult to assign a definite number to the tepals which compose the perigon, although it is probably four. The vascular system of the floret proper is much dissected because of the many anastomoses from the tertiary vascular axis (fig. 112) which form the supply to the tertiary bracts. An

inner set of anastomoses arises simultaneously to form the small cylinder responsible for the formation of the ventral bundle. From this cylinder a third set of anastomoses again passes outward to refuse with traces persisting after the departure of the tertiary bract bundles to form the perigon supply. Because of the discrete nature of the supply to the tertiary bracts and the absence of the secondary bracts it is difficult to assign a definite orientation to the ovary, but from a study of the younger stages, before increase in size of the vascular system, it seems clear that the ovary is diagonal.

The ventral bundle divides somewhat below the insertion of the two ovules, which are on diagonally opposite margins of the two carpels in this ovary. The number of traces in the perigon is much greater than it would be if each of the perigon segments were supplied with but three bundles. This may be explained by the extreme dissection of the vascular system in the much abbreviated tertiary axis and the great increase in size of the ovary over that usual in the family. Thus a greater degree of dissection of the vascular supply to the individual tepals than that observed elsewhere in the family may have occurred.

*Alnus, pistillate florets* (see footnote 2)

The individual florets ("pistils") in this genus present a delusive appearance of simplicity. Examples of these from widely differing species are shown in figures 38 and 44. An occasional species such as *A. subcordata* (fig. 41) has as appendages to the small flattened "pistil" two or more glands at the base of the stylar column. These glands are especially well developed in this species, the presence of four being unusual for the genus, although common in this species. The glands uniformly occur one opposite each of the dorsals of the ovary and one on either side opposite the septum. In other words, these glands are in the positions which would be occupied by tepals in a tetramerous perigon. Furthermore, minute but recognizable vascular bundles are sometimes to be found in the outer portion of the ovary wall in the positions they would normally occupy if there were four such perigon segments fused to the ovary. In most species of the genus these glands are much smaller and opposite the dorsals only, or may be absent completely. However, in one lot of material

of *A. rubra*, otherwise normal, there are definite free or partially fused tepals. In this case, which will be further described in the next contribution of this series, vascular bundles are regularly associated with the partially free tepals. On the other hand, in *A. incana*, with only small glands or none at all, occasional vestigial bundles are present in the basal portion of the ovary, bundles which are evidently homologues of those supplying the poorly developed or vestigial perigon segments of other species.

Aside from the weakly developed or completely suppressed tepal supply, the vascular system of the florets is simple. The traces composing the tertiary vascular system combine loosely to form a diminutive pedicellar siphonostele (figs. 13, 30, 55) from which depart almost immediately the two dorsal bundles (figs. 14, 30, 57). The remaining vascular tissue combines to form the ventral fusion bundle (figs. 31, 58). In several species this bundle divides into two pairs of three bundles each (*A. crispa* var. *mollis*, fig. 31; *A. incana*, fig. 61), each set of three supplying an ovule. Beyond this point there is no extension of the ventral bundle. The septum breaks down immediately after the departure of the vascular tissue to the ovules (figs. 33, 34), forming a fusion loculus (figs. 34, 35, 62) which is continuous with the interstylar canal. On the whole the vascular system of the ovary is identical with that of the other members of the family, except for the drastic reduction of the perigon, correlated doubtless with the great reduction in size of the floret.

The ovaries at first appear to be median to the secondary bracts but upon closer observation are clearly diagonal, slightly modified by the pressure of adjacent cymules.

*Betula, pistillate florets* (see footnote 5)

Morphologically the florets of this genus are much like those of *Alnus*. No cases were found of tepals as well defined as they are in some species of *Alnus*. Glands similar to those present in some species of *Alnus* also appear sporadically in this genus, as in *B. coerulea-grandis* (fig. 98′) and in *B. maximowicziana*. In no case have vascular elements been found associated with these glands as in some species of *Alnus*, there being present only elongate hyaline rows of cells approaching the insertion of the gland at the base of

the stylar column. The homology of the glands in *Alnus* and *Betula* is shown by their great similarity, indicating that in *Betula* these glands also suggest the former presence of foliose tepals.

The vascularization of the florets is also much like that of the florets in *Alnus* although somewhat simpler. There is much greater reduction of the vascular tissue composing the tertiary vascular axis, and it is not cylindrical as in *Alnus*. Thus there is simply a branching of the vascular tissue to make up the dorsals on the one hand and the ventral on the other. The dorsals follow the usual course, as does the ventral. In the case of the ventral, however, it simply bifurcates to supply the two ovules, which may be on adjacent or diagonally opposite carpel margins. The septum of the ovary does not ordinarily break down until after the departure of the ovule bundles, and there follows the formation of a fusion loculus, ultimately continuous with the interstylar canal. Occasionally tricarpellary ovaries occur, as in *B. luminifera* (fig. 86).

The tertiary florets have their ovaries "medianly" (diagonally) oriented with respect to the secondary bract, while the secondary floret has its ovary transverse to the primary bract.

### Summary of floret morphology and anatomy

It is clear from the uniform presence of a perigon in the pistillate florets of the Coryleae, and the occurrence of a perigon in the staminate florets of the Betuleae, that the members of the family must be considered as derived from some ancestor which possessed perigon segments regularly in the florets of both sexes. Furthermore, vestiges of a perigon are found occasionally in the staminate florets of the Coryleae and in the pistillate florets of the Betuleae, suggesting reduction of the perigon in these forms.

In the less reduced staminate florets a hexamerous condition is characteristic, while in the pistillate florets trimerous pistils occur in almost every genus. If these indications are significant then we must hypothecate for the family as a whole an ancestral condition in the phylogenetically recent past in which the florets were trimerous throughout, as shown in the floral diagrams in figures 5 and 6. Under such circumstances we are in a position to evaluate the puzzling differences in orientation of the pistils in the various genera of

the family. The diagonal orientation of the pistil in the tertiary pistillate florets of *Ostryopsis* (fig. 100), *Alnus* (fig. 39), and *Betula* (fig. 77) may well be associated with the suppression of the adaxial diagonal carpel of a tricarpellary ovary (fig. 5). On the other hand the transverse pistils of *Carpinus* (fig. 152) and *Ostrya* (fig. 123) are doubtless associated with the suppression of the median carpels of the tertiary florets (fig. 5). There remains the condition in *Corylus* (fig. 120) in which the ovaries of the tertiary florets appear to be median to the secondary bracts, but this seems to be associated with a slight displacement of ovaries originally diagonal.

In the pistillate cymules of *Betula* there is the apparent anachronism of diagonal pistils in the tertiary florets and a transverse pistil in the secondary floret (fig. 77). This, of course, may be explained either by the suppression of the median carpel in the secondary floret and also of the adaxial carpel in the tertiary florets, or by the suppression of one of the diagonal carpels in the secondary florets and a later twisting of the floret to bring the pistil into a transverse position. The answer to this problem awaits the solution of the question whether the florets of *Betula* became dimerous after the effects of crowding in the ament had been reflected in the morphology of the florets, or whether this reduction to dimery came earlier. Either point of view is tenable.

In the staminate florets there is a remarkable parallel series in the reduction of the androecium in *Alnus* and *Betula* on the one hand and in *Carpinus*, *Ostrya*, *Ostryopsis*, and *Corylus* on the other hand. In the former the reduction series is accompanied by a corresponding reduction in the perigon and in the latter by a complete absence of the perigon, with the possible exception of *Corylus*. In both series there is also a tendency toward separation of the anthers, a tendency which goes much further in the latter series than in the former.

## Literature review

The following significant questions arise in reviewing the literature on the morphology of the inflorescence and florets.

### 1. Interpretation of primary, secondary, and tertiary bracts

Up to the time of Hartig (1852) and Döll (1843) the bracts were considered as simply scales or appendages, or were even confused with tepals, although the latter misconception was early recognized as such by Fant (**12**). With the increasing tendency to homologize the foliar organs with one another under the influence of the metamorphosis theory of Goethe (**13**), two schools of thought arose, the one founded by Hartig (**15**) and the other by Döll (**8**). Hartig's stipular theory to account for certain of the bracts has been variously modified and finds a recent exponent in Pauchet (**20**). On the other hand, Döll's (**9**) theory of the strictly laminar nature of the different bracts has attracted a tremendous following (**22, 11, 23, 1, 35, 36, 26,** and most other contemporary morphologists and systematists). Since, however, the stipular theory is so ingenious in certain of its forms (**33**), it is well to review the anatomical evidence against it. A single example will suffice. In the staminate cymules of *Carpinus japonica* it was found that those from the base of the ament had, instead of primary bracts, a reduced lamina flanked by stipules (fig. 241), and that secondary bracts were present as well. The vascular supply to these stipules is derived as branches departing laterally from the laminar supply subsequent to its becoming free from the primary vascular cylinder. The vascular supply to the secondary bracts is derived from the secondary axis cylinder in the usual fashion, and is distinct in every way from that to the reduced lamina and its stipules. Passing to a cymule in which the primary bract is typically developed and is accompanied by secondary bracts (fig. 239), the superficial resemblance of this complex of bracts suggests that the secondary bracts may represent the stipules of a reduced leaf of which the primary bract represents the lamina. That this is not the case is evidenced by the complete lack of relationship between the vascular supply of the primary and the secondary bracts, since the primary bract derives its supply from the base of the gap in the primary vascular axis while the secondary bracts receive their vascular supply from bundles departing laterally from the secondary vascu-

lar axis. Had these secondary bracts been stipules, as the adherents of the stipular school would hypothecate, then their vascular supply would have consisted of branches from the primary bract bundle. Throughout the family, in the modified leaves entering into the composition of the cymule there is no evidence of deviation from the anatomical pattern characterizing the vascular origin of successive exstipulate leaf organs on a shortened axis. DÖLL'S theory is fully substantiated by the anatomical evidence.

The absence of secondary bracts in the pistillate cymules of *Corylus* has been noted by only two workers, SCHACHT (**27**) and VAN TIEGHEM (**33**). That this important fact has been overlooked is doubtless due to the superficial resemblance between the tubular involucre of some species of *Corylus* and that of *Ostrya*, which because of its evident close relationship to *Carpinus* has been considered to have both secondary and tertiary bracts. SCHACHT and VAN TIEGHEM found, by careful ontogenetic study of species of *Corylus* in which the involucre is composed of free members, that three bract primordia were present in the early stages of development, but that the one occupying the position of the secondary bract very early ceased developing while those in the position of the tertiary bracts persisted to form the involucre. The vascular system of the cymule has been considered in detail in this paper and substantiates the conclusions reached earlier by SCHACHT and VAN TIEGHEM.

### 2. NATURE OF STAMINATE INFLORESCENCE IN THE CORYLEAE (CARPINUS, OSTRYA, CORYLUS, AND OSTRYOPSIS)

Almost without exception the staminate ament of the Coryleae has been interpreted as a spike, the implication being that each primary bract subtends but a single floret. As early as 1851, however, WYDLER (**37**) suspected that the staminate ament in this group was composed of a series of cymules, since he found the number of stamens in each cymule to be about three times as many as they were in each of the three florets of a corresponding cymule in the Betuleae. Furthermore he pointed out the divided nature of the stamens in the Coryleae, which helped clarify the morphologi-

cal concept of the time. His idea was put forward only as a suggestion without proof, a form in which EICHLER (11) gave it his sanction. The concept has lain dormant but is evidently in need of revival because the vascularization of the cymules indicates clearly the correctness of WYDLER's intimation.

### 3. NATURE OF THE OVARY: AXIAL OR APPENDICULAR

Many workers from the time of SCHACHT (27) have recognized the foliar nature of the stigmas of the pistils of the Betulaceae, but interpretation of the nature of the ovary wall has been varied. Thus SCHACHT, following PAYER's (21) general line of reasoning, concluded that the ovary wall in the Betulaceae is axial, a point of view adopted in a modified form by NAVASHIN (19) and STREICHER (31). Opposed to this interpretation of the ovary wall in flowering plants in general are VAN TIEGHEM (32), HENSLOW (16), and EAMES (10). The latter two consider the ovary wall to be foliar even though it may arise as it does in the Betulaceae through the intercalary growth of a ring of tissue rather than from as many distinct "anlagen" as there are carpels in the ovary. In no way does the vascular anatomy of the ovaries of the Betulaceae support the notion that either the walls or the placentae are axial, since the vascular supply to the carpel walls arises at the base of the ovary in a manner like that of a reduced leaf and bears no similarity in behavior to the vascular tissue in stems. Thus the arguments brought forward by WOLPERT (36) in favor of the appendicular nature of the ovary and placentae in this family are fully supported by the anatomical evidence.

### 4. SUPERIOR OR INFERIOR CONDITION OF OVARY IN THE BETULEAE

The great majority of the students of the Betulaceae have accepted the ovary of the species in the subfamily Betuleae without question as being superior, or when in doubt, have referred to it as nude (BENTHAM and HOOKER 3). DÖLL (8), and later WOLPERT (36), have suggested that the ovary in this group has fused to it a rudimentary perigon. They employ one of the four lines of evidence which lead to this conclusion. This is the presence at the base of the

stylar column of glandular structures resembling those present on the margins of the tepals in the staminate florets (fig. 41). Other lines of evidence of which they did not avail themselves are the following. First, in the ontogenetic field, where it has long been a matter of common knowledge that the inferior ovary is distinguished from the superior ovary by arising through the basal growth of a ring of tissue (**21, 7**). In the Betuleae the ovary wall forms as a result of the basal growth of a ring of tissue (**19**), evidence that the ovary is essentially inferior. Second, as has been pointed out in the preceding pages, occasional vestigial bundles occur in the walls or at the base of the ovary in species of *Alnus*. These bundles are in the position occupied by the major bundles of the perigon in genera of the Betulaceae where a perigon is undoubtedly present. Third, there has been observed in a single collection of *A. rubra* a perigon composed in part of free and in part of fused tepals. While this may be considered teratological, and therefore perhaps of secondary importance, the other three lines of evidence support the validity of this particular evidence. While it is not intended to state dogmatically that a perigon is present in all species of the Betuleae, for it is not reasonable to say that a structure is present if it has been lost, it is nevertheless suggested that the pistil of the Betuleae be recognized as inferior, and representative of a much reduced floret. The only other interpretation possible is that the perigon is arising anew, an idea unacceptable in the light of Dollo's law.

### 5. Misconceptions and unsolved problems

Perhaps the most striking misconception is that concerning the morphology of the florets in the species of s. Clethropsis in *Alnus*. Spach (**29**) described the staminate cymules as uniflorous with 10- to 12-parted florets. The idea has persisted in the literature essentially unchallenged except by Eichler (**11**), who suggests that there may be a three-flowered cymule here. That this is actually the case has been pointed out in the foregoing pages. The error was doubtless due to the discrepancy in size between the diminutive tepals and the long-filamented stamens, together with the paucity of the material, since Spach (**29**) made a similar error in the interpretation of the cymule of the species of s. Alnobetula of *Alnus*. In the latter case an

abundance of flowering material was readily available in Europe, and the mistake was corrected by SPACH'S successors.

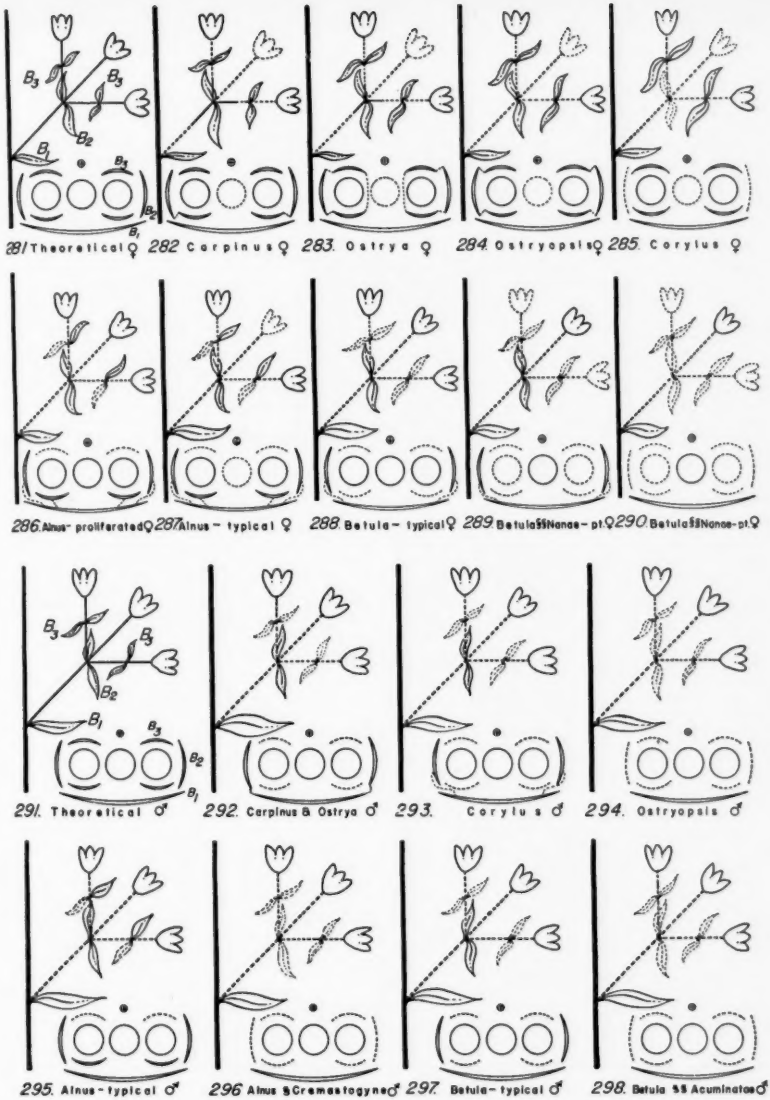
The amazing suggestion made by DE CANDOLLE (**4**) that the florets of the staminate cymules in the Betulaceae are simply proliferations on the upper surface of the complex of bracts deserves comment primarily for its eccentricity, and indicates to what extremes a purely ontogenetic study may lead. If the concept of the flower as a short shoot is to be abandoned, as VUILLEMIN (**34**) and GRÉGOIRE (**14**) would have us believe, and if the inflorescence is not a branch system as DE CANDOLLE implies, then there is no sound basis left for the study of comparative floral and inflorescence morphology.

A major problem, first recognized by DÖLL (**8**), is the variation in the orientation of the pistil from genus to genus. Two factors are to be considered. One is the effect of the vigorous crowding together of organs in the ament. Doubtless it is this which is responsible for the absence of the secondary florets in all the genera of the family except *Betula*. In *Betula* this factor may be responsible for orientation of the secondary floret ovary transverse to the primary bract whereas the ovaries of the tertiary florets are roughly median to the secondary bracts. For conclusive evidence we must resort to experiment, but, in the absence of this, the circumstantial evidence to be derived from the presence of secondary florets in the proliferated material of *Alnus* is instructive. Here it is found that in the absence of a strong dorsiventral pressure the secondary floret has its ovary disposed medianly to its bract, thus indicating, by analogy at least, the effect of absence of pressure on the orientation of the ovary of the secondary floret.

The second factor involved in a consideration of the orientation of the ovaries is that dealing with the occasional presence of tricarpellary ovaries in the family. While this will be described at greater length in the next paper of this series, it suffices to state that in every genus studied tricarpellary ovaries have been found in one or more species, often occurring in otherwise normal aments. In the light of Dollo's law it is not unreasonable to suppose that tricarpellary ovaries were once more prevalent in the florets of the family than they are today. Many observers from the time of

SPACH (30) have noted the occasional presence of tricarpellary ovaries. Furthermore, supporting evidence is to be derived from the staminate florets, since they are often hexamerous; and when not hexamerous, WOLPERT (36) has pointed out that these florets may begin with the *anlagen* of a hexamerous condition, only to have some of the *anlagen* suppressed in later development. Granting the possibility that the trimerous gynoecium (floral diagram in fig. 5) was ancestral and the dimerous gynoecium derived, there is the further possibility that the tertiary florets have become bicarpellary by the loss of either the carpel in the plane of the secondary bract or one of the carpels in the plane of the tertiary bracts. In *Carpinus*, *Ostrya*, and *Ostryopsis* the vascularization of the gynoecium indicates that the carpel in the radius of the abaxial tertiary bract is present, because its dorsal arises in the same radius as does the bundle to the abaxial tertiary bract. The dorsal of the other carpel, however, arises in *Carpinus* and *Ostrya* in the radius of the adaxial tertiary bract trace, while in *Ostryopsis* it arises in the radius of the secondary bract trace. The result is the orientation of the ovary transverse to the secondary bract in *Ostrya* and *Carpinus*, and a "median" (or more properly, diagonal) orientation of the ovary to the secondary bract in *Ostryopsis*. In *Alnus*, *Betula*, and *Corylus* the changes imposed by extreme smallness or largeness of the ovaries on the vascular systems have obscured the relationships of the dorsals of the ovaries to the bract traces; but it is evident that essentially they share with *Ostryopsis* the diagonal orientation of the ovaries, which has erroneously been considered median to the secondary bracts.

The staminate florets in the Betuleae provide no problem in interpretation, since forms which are reduced to monomery, such as those which occur in the ss. Nanae of *Betula* or in the s. Cremastogyne of *Alnus*, are connected by a continuous series of intermediate stages with morphologically orthodox hexamerous florets. They are also provided with vascular systems whose characteristics are in accord with the preceding interpretation.


281 Theoretical ♀
282 Carpinus ♀
283. Ostrya ♀
284 Ostryopsis ♀
285. Corylus ♀
286. Alnus - proliferated ♀
287 Alnus - typical ♀
288 Betula - typical ♀
289 Betula §§ Nanae - pt. ♀
290 Betula §§ Nanae - pt. ♀
291. Theoretical ♂
292. Carpinus & Ostrya ♂
293. Corylus ♂
294. Ostryopsis ♂
295. Alnus - typical ♂
296 Alnus § Cremastogyne ♂
297. Betula - typical ♂
298. Betula §§ Acuminatae ♂

FIGS. 281–298.—Figs. 281–290, diagrams to show presence (solid lines), absence (broken lines), and fusion (dotted lines) of components of more common types of pistillate cymules in Betulaceae. Figs. 291–298, diagrams to show presence (solid lines), absence (broken lines), and fusion (dotted lines) of components of more common types of staminate cymules in Betulaceae.

**Summary**

1. The anatomical method provides a key to the interpretation of floral and inflorescence morphology in the Betulaceae. The floret has the anatomical characteristics of a short shoot and undoubtedly is of that nature. Likewise on an anatomical basis the inflorescence is a branch system.

2. The morphology and anatomy both of the cymules and of the florets have been notably modified by dorsiventral or lateral concrescence, shortening of internodes, pressures within the ament, and reduction.

3. The bicarpellary ovaries, either transverse or diagonal to the secondary bract, owe their diverse orientation to their derivation from an ancestral tricarpellary ovary. The staminate floret in many species still has a trimerous groundplan.

4. On the basis of these factors, the morphology of the cymules and florets of the Betulaceae may be interpreted (figs. 281–298) as follows:

a. The staminate cymule is three-flowered in all genera of the family. The pistillate cymule is two-flowered by suppression of the secondary floret in all genera except *Betula*, in which all three florets are present.

b. The full complement of bracts is present in the pistillate cymules of *Carpinus*, *Ostryopsis*, and *Ostrya*. The adaxial tertiary bract is lost from the staminate and pistillate cymules of most species of *Alnus*. Both adaxial and abaxial tertiary bracts have been lost from the staminate cymules of *Betula*, *Corylus*, *Carpinus*, *Ostrya*, and from the pistillate cymules of *Betula*. The secondary bracts have been lost while the tertiary bracts persist in the pistillate cymules of *Corylus*. All bracts but the primary have been lost in the staminate cymules of *Ostryopsis*.

c. The perigon is present in the pistillate florets of *Carpinus*, *Ostrya*, *Corylus*, *Ostryopsis*, and in the staminate florets of *Alnus* and *Betula;* it is obsolescent in the pistillate florets of *Alnus* and *Betula;* it is completely lost from the staminate florets of *Carpinus*, *Corylus*, *Ostrya*, and *Ostryopsis*. The ovary is inferior throughout the family.

d. The phylogenetic loss of the carpel in the radius of the secondary bract has resulted in the transverse bicarpellary ovary of *Carpinus* and *Ostrya*. The loss of the carpel in the radius of the adaxial tertiary bract has resulted in the so-called "median" (or more properly, diagonal) bicarpellary ovary of *Alnus*, *Betula*, *Corylus*, and *Ostryopsis*.

I wish to express my appreciation to Professor A. J. EAMES of Cornell University for his recommendation of a study of the floral anatomy of this family, for the privilege of working on the staminate material in his laboratory, and for his good counsel in the course of the investigation. I wish to express my gratitude to Professor R. H. WETMORE of Harvard University for his keen and constructive contributions while I enjoyed the privilege of working in his laboratory on the pistillate anatomy and the other phases of the problem introduced at that time. I owe thanks to Professor I. W. BAILEY of the Arnold Arboretum for his stimulating suggestions and for the opportunity of studying the collection of wood slides in his laboratory. To my wife, LUCY B. ABBE, I am indebted for critical collaboration and technical assistance throughout the course of the investigation.

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# INFLUENCE OF SULPHUR DEFICIENCY ON THE METABOLISM OF THE SOY BEAN

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 461

SCOTT V. EATON

(WITH TWO FIGURES)

## Introduction

In an earlier publication (7) attention was called to the rather peculiar position of sulphur in the list of elements essential to the growth of plants. Ever since the now obsolete ten-essential-elements concept was established, sulphur has been a member of the list, although it has not been considered necessary to use sulphur fertilizers. One of the main reasons for this has probably been that the early ash-analysis method of determining sulphur in plants showed such a small amount present that it was thought that the supply in the soil plus that brought down by the rain was ample for the needs of crops. When later accurate fusion methods of analysis showed much more sulphur present than the ash analysis indicated, the whole question of the need of fertilizing crops with sulphur was reopened.

Most of these early investigations were carried on by agricultural experiment station workers, who were primarily interested in the practical aspects of the question. Numerous papers appeared dealing with such subjects as the sulphur content of soils, the amount of sulphur brought down by the rain, the sulphur content of crops, and the amount of sulphur in drainage water. But it was recognized that, after all, the best way to determine whether an element is deficient in the soil is to employ fertilizers containing this element and to see whether the yield is thereby increased. A number of workers investigated this question in connection with sulphur.

Of the agricultural soils of the United States, those of the northwest have been found especially deficient in sulphur (32, 26). A later contribution to the soil sulphur literature is that of POWERS (30), and recently STOREY and LEACH (34) showed that the disease of the tea bush known as yellows, which has seriously affected the growing

of tea in Nyassaland, Africa, is due to a deficiency of sulphur in the soils of this region.

Most of the investigations on sulphur having to do with plants have dealt primarily with its effect on crop yield, although some of the external effects have been noted, such as effects on color, size of plant, root development, and nodule development of the roots (**26, 32**). Also, some noted a higher nitrogen content of plants fertilized with sulphur (**26, 32**) and some divided the total sulphur of the plants into fractions (**20, 32**). But there has been lacking a thorough study of the effects of sulphur deficiency on the metabolism of the plant, such as has been supplied for the tomato by the recent work of NIGHTINGALE (**22**).

Before NIGHTINGALE'S paper appeared the writer had started investigations on the effects of sulphur deficiency on the growth of plants. Preliminary experiments were performed in the spring of 1930. Most of this early work was concerned with the choice of a plant, several kinds being grown in washed quartz sand, including soy bean, alfalfa, rape, kale, mustard, wheat, and oats. Soy bean was finally selected.

## Methods

### GROWING THE PLANTS

PRELIMINARY EXPERIMENTS WITH SOY BEAN.—Considerable difficulty was experienced in demonstrating marked sulphur deficiency in the soy bean. The chief trouble was that the plants blossomed before any decided symptoms of sulphur deficiency appeared, and when the soy bean blossoms vegetative growth largely ceases. In the spring of 1932 Wisconsin Black soy bean plants were grown in pure quartz sand. Some of the plants were given a complete nutrient solution and some a solution lacking sulphates. The first blossoms appeared on June 20, after about four weeks of growth. The minus sulphur plants showed some symptoms of sulphur deficiency but they were not very marked. The plants were harvested on June 24 and analyzed for various carbohydrate and nitrogen fractions. Since the differences between the plus and minus plants were small, the results are not given.

It was decided to try to prolong the vegetative period so that the

effects of omitting sulphates from the nutrient solution would be greater. This was first attempted by increasing the nitrate content of the nutrient solution. In the fall of 1932 three sets of soy bean plants were grown in quartz sand with a nutrient solution the nitrate content of which was low in one set, medium in another, and high in the third. Each set was divided into two groups, one group receiving sulphate and the other receiving none. The vegetative period was prolonged somewhat by this treatment, but not markedly. The plants blossomed at about the normal time and again before marked symptoms of sulphur deficiency were obtained, although the minus sulphur plants were somewhat less green and had somewhat smaller leaves and thinner stems than the plus sulphur plants. It was perhaps hardly to be expected that the vegetative period would be much prolonged by this treatment, because of the short days of the autumn, many of which were cloudy. ECKERSON (11) has shown that poor light conditions cause a low reducase content of the Biloxi soy bean. Increasing the nitrate content of the nutrient solution under these conditions has little effect in increasing the growth of the plant or in prolonging the vegetative period, for the nitrates are not being used. The results might be different in the spring.

Attempts were next made to keep certain groups of plants in each of the nitrate series free of buds by picking them off as fast as they appeared. This was not very successful. Removing the buds tended to prolong the vegetative period somewhat, and the minus sulphur plants showed to a certain degree some of the characteristic symptoms of sulphur deficiency. But removing the buds caused them to be formed in even greater profusion, so that it was impossible to keep the plants entirely free of buds. Under these conditions uniform material for analysis could not be obtained.

It was found in previous work (8) that if the natural day length was prolonged to about 16 hours by using electric lights, soy bean plants did not blossom in about $5\frac{1}{2}$ weeks. It was decided to use this method, although it was recognized that it was subjecting the plant to somewhat abnormal conditions.

FINAL PLAN.—Soy bean plants of the Manchu variety were grown in pure no. 3 quartz sand in 2-gallon glazed earthenware crocks provided with a hole in the bottom for drainage. The seed was given to

the writer by Dr. E. W. HOPKINS, who received it through the kindness of the Department of Agronomy of the University of Wisconsin. The sand was washed thoroughly with distilled water. The plants were grown without nodule development, and to insure that no nodules would develop the seed was sterilized with 0.25 per cent uspulun solution and the sand was sterilized in a soil sterilizer. The seeds were selected for uniformity, and an excess of seeds was planted directly in the sand. About two weeks after the seeds had sprouted, the seedlings were thinned to five uniform plants per pot. Four 1000 watt Mazda bulbs were placed about 2 feet above the plants. When daylight became somewhat weaker in the afternoon these lights were turned on and left on until 10 P.M. The plus sulphur series of 1933 consisted of 187 plants and the minus sulphur, 190; the numbers in the 1934 experiment were 104 and 106 respectively.

The plants were given a nutrient solution that had been found in previous work (**8**) well suited for the growth of the soy bean. Certain modifications were made for this work. Since the plants were grown without nodule development, the nitrate content was higher than in the previous work. Solution A of the formula consisted of molecular calcium nitrate. In the case of the solution lacking sulphate, magnesium chloride in such an amount as to give the same amount of magnesium was substituted for the magnesium sulphate. The plants grown in the spring of 1934 received a somewhat more concentrated nutrient solution during the latter part of the growth period, when they were larger. The pH of the nutrient solution used in 1933 was not controlled; that used in 1934 had an initial pH of 4.6–4.9. The nutrient solution was sprinkled on the surface of the sand. It was applied five days each week, enough being added so that a considerable amount ran out through the hole in the bottom of the crock. On the other two days of each week the sand was thoroughly flushed with distilled water.

It was found necessary to supply the plants with a little boron. A number of the plants grown in 1933 had dead tips after about six weeks' growth. Dr. NIGHTINGALE, who was working in our laboratory at the time, said that this characteristic injury was no doubt due to boron deficiency (**2, 19**). As soon as the injury was noted boron was added twice each week at a concentration of 0.5 p.p.m.,

and as an additional precaution manganese was also applied at a concentration of 0.25 p.p.m. They were added with the distilled water used to flush the sand. In a short time shoots were developed from the axillary buds. In the case of the 1934 experiment, boron and manganese each at a concentration of 0.25 p.p.m. were applied twice each week throughout the period of growth, and no boron injury developed.

The soy bean plants were grown in the greenhouse in the springs of 1933 and 1934. It was impossible to control accurately the temperature and humidity. There was, of course, considerable variation in these conditions. In 1933 the seed was planted on March 7. Two samplings were made, one on April 22 and the other on May 5. In 1934 the seed was planted on March 1 and the plants were harvested on April 21. In 1934 sunflower, white mustard, kale, and rape were also grown, using the same nutrient solution and method that were used in growing the soy beans. The seed was planted on February 26 and the plants were harvested on April 27. No analyses were made.

## Chemical and microchemical methods

Sampling and extraction.—The method of sampling and extraction was essentially that described in a previous publication (**8**), except that in the present work leaves, stems, and roots were sampled separately, and the material was cut into fine pieces (**21**) instead of being ground in a mill. In the case of the plants grown in 1934, a composite sample of the stem tips, petioles, and axillary buds was made, so that the chemical composition of the entire plant could be estimated. The samples were extracted with hot 80 per cent alcohol, the alcohol being boiled each time on the steam bath for 15 minutes. The extract was allowed to cool to room temperature before filtering. Four or five extractions were made. It would probably have been better to have used a somewhat lower concentration of alcohol for the extraction, for it was a little difficult in the case of the samples of the minus sulphur stems to avoid the precipitation of a few crystals which seemed to be amide nitrogen. There was a little loss of this fraction in these samples, but not enough to affect the results materially. As shown by table II, the minus sulphur stems were very high in amide nitrogen as compared with the plus sulphur stems.

CARBOHYDRATE FRACTIONS.—The water extract for the sugar determinations was prepared as previously described (**8**). The sugars were determined by a combination of the Munson and Walker and Bertrand methods (**18**). Acid hydrolysis was used in the determination of total sugars. The solution was neutralized with 50 per cent sodium hydroxide, the exact end point being adjusted with 0.1 N acid and alkali. Phenolphthalein was the indicator used.

The residue was dried to constant weight, ground first in a coffee mill and then in a ball mill, so that it would pass through a 100 mesh sieve, and again dried to constant weight. Starch was determined in the powdered residue by salivary digestion by the method of NIGHTINGALE (**21**), the method being modified somewhat. The residue was boiled with water for only 3–5 minutes. It was then digested at 80° C. in an oven for 30 minutes. After salivary digestion, the extract was heated in a boiling water bath for 2.5 hours with 2.5 per cent HCl. After neutralization, the extract was cleared and deleaded before the reduction was made. Hemicelluloses were determined by the method of CLEMENTS (**3**), except that hydrochloric acid was used in place of sulphuric acid. The extract was cleared and deleaded as in the case of the starch extract. In both cases the Bertrand modification of the Munson and Walker method was used to determine the resulting sugar.

NITROGEN FRACTIONS.—Ammonia nitrogen, nitrate nitrogen, and amino nitrogen were determined by the methods of PHILLIPS (**28**). The reduced iron method of PUCHER, LEAVENWORTH, and VICKERY (**31**) was used for the determination of total nitrogen in the extract. The method was followed exactly in the reduction process. For the digestion process, copper sulphate was used as the catalyst instead of mercury, and potassium permanganate was not added. Sodium thiosulphate was not added before the distillation, of course. Total nitrogen in the residue was determined by the Gunning method (**1**). Total organic nitrogen was obtained by subtracting nitrate nitrogen from the total nitrogen; soluble organic nitrogen, by subtracting nitrate nitrogen from the total nitrogen of the alcoholic extract. Since 80 per cent alcohol was used for the extraction, this fraction is not the same as in the case of the water extraction method.

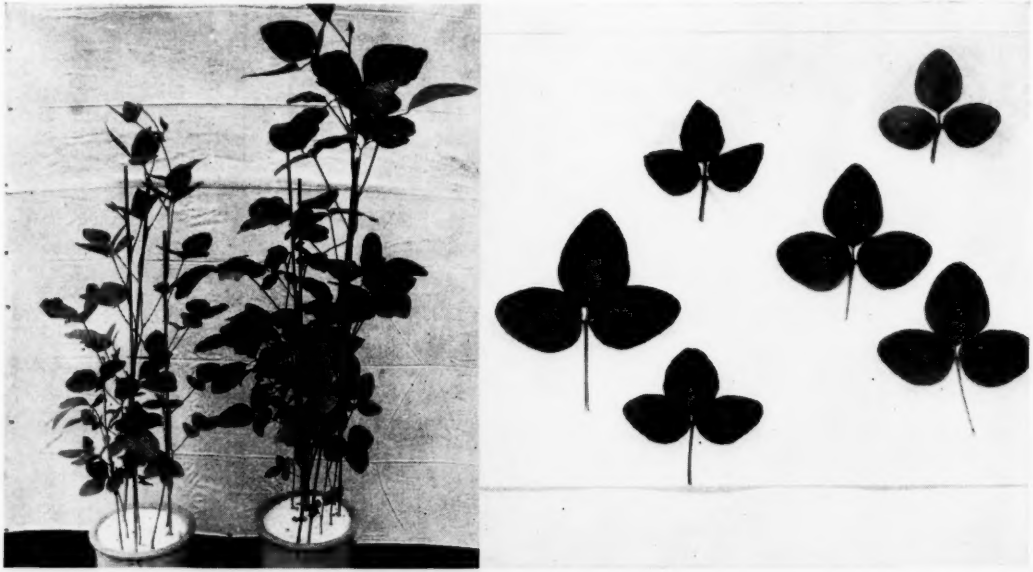
SULPHUR FRACTIONS.—An attempt was made to determine volatile sulphur in green, finely ground soy bean tissue by PETERSON'S

method (**27**) in order to determine whether there was danger of losing any sulphur on drying the residue to constant weight. The method did not prove to be very satisfactory, but the trials indicated the presence of such an extremely small amount of volatile sulphur that it was not considered worth while to determine this fraction. Total sulphur and sulphate sulphur were therefore determined in the powdered residue and in the extract after evaporating off the alcohol by the official methods (**1**). For the sulphate determination on the residue a water extract was made, and this extract as well as the alcohol-free extract was freed of coagulable material by heating to boiling, adding acetic acid to the hot solution, and filtering. Organic sulphate represents the difference between total sulphur and sulphate sulphur.

MICROCHEMICAL METHODS.—Microchemical tests were made on the green tissue for nitrates, reducing sugars, starch, and sulphates by the methods of ECKERSON (**10**).

## Data

EXTERNAL EFFECTS OF SULPHUR DEFICIENCY.—It took considerable time for the symptoms of sulphur deficiency to develop, but in four weeks they were beginning to show, and in six weeks they were pronounced. They were somewhat more marked in the plants grown in 1933 than in those grown in 1934, but were similar in the two sets. The main symptoms were the yellow-green color of the leaves, the smaller leaflets, and the thinner stems of the sulphur deficient plants as compared with the plus sulphur plants (fig. 1). Also the stems of the former were harder than those of the latter. It was repeatedly noticed that the upper leaves of the sulphur deficient plants (not counting the small undeveloped leaves) became yellow first (fig. 2). There was increasing greenness from the top to the bottom of the plant. In certain cases the lower leaves also became yellow, but this was attributed to the breakdown of the chlorophyll incident to the death of the leaves. There was a tendency for the lower leaves, especially in the case of the minus sulphur plants, to die and fall off. The difference in size of the leaves of the plus sulphur and minus sulphur plants was pronounced. Some of the 1933 plants were allowed to grow after the last sampling was made. On May 29, four



FIGS. 1, 2.—Fig. 1 (left), soy bean plants (May 5, 1933): minus sulphur plant on left; plus sulphur plant on right. Fig. 2 (right), soy bean leaves arranged according to position on plant (April 18, 1933): plus sulphur leaves on left; minus sulphur leaves on right. Photographs by E. W. HOPKINS.

leaves from the tallest sulphur deficient plant and four leaves from corresponding levels on the tallest plus sulphur plant were blue printed and the area determined with a planimeter. The area of the minus sulphur leaves totaled 371.8 sq. cm.; that of the plus sulphur leaves, 786.3 sq. cm.

TABLE I

GREEN WEIGHT (100 PLANTS), PERCENTAGE MOISTURE, PERCENTAGE DRY WEIGHT, TOP-ROOT RATIO

| | Samples taken | | | | | |
|---|---|---|---|---|---|---|
| | April 22, 1933 | | May 5, 1933 | | April 21, 1934 | |
| | Plus S | Minus S | Plus S | Minus S | Plus S | Minus S |
| Green weight (gm.) | | | | | | |
| Tops | 557.2 | 439.4 | 1280.8 | 786.6 | 1364.9 | 873.9 |
| Leaves | 239.5 | 180.2 | 533.3 | 326.0 | 507.4 | 351.4 |
| Stems | 213.6 | 186.0 | 470.2 | 294.1 | 550.0 | 350.7 |
| Roots | 231.1 | 214.0 | 580.0 | 516.3 | 506.9 | 500.1 |
| Entire plant | 788.3 | 653.4 | 1860.8 | 1302.9 | 1871.8 | 1374.0 |
| Top-root ratio | 2.411 | 2.052 | 2.208 | 1.523 | 2.730 | 1.747 |
| Percentage moisture | | | | | | |
| Leaves | 79.03 | 78.52 | 79.42 | 78.48 | 82.32 | 81.51 |
| Stems | 81.05 | 78.49 | 81.08 | 79.99 | 83.44 | 83.05 |
| Roots | 90.63 | 91.11 | 92.32 | 92.78 | 90.88 | 91.47 |
| Percentage dry weight | | | | | | |
| Leaves | 20.97 | 21.48 | 20.58 | 21.52 | 17.68 | 18.49 |
| Stems | 18.95 | 21.51 | 18.20 | 22.10 | 16.56 | 16.95 |
| Roots | 9.37 | 8.89 | 7.68 | 7.22 | 9.12 | 8.53 |

Stem elongation and the twining habit were prominent features of both series of plants. Of course, because of the use of electric lights the plants of neither series were normal (**8, 29, 38**). But omitting sulphur from the nutrient solution did not prevent stem elongation, although the minus sulphur plants were on the average not so tall as those grown with a complete nutrient solution (fig. 1). On April 19, 1934, when the plants were about seven weeks old, three of the tallest minus sulphur plants and three of the tallest plus sulphur plants were measured. The three minus sulphur plants measured 34, 34.5, and 34.75 inches in height; the three plus sulphur plants,

33·75, 36, and 37·75 inches. The stems of the sulphur deficient plants were decidedly smaller in diameter throughout their entire length than the stems of the plants grown with a complete nutrient solution, being in some cases less than 2 mm. in diameter at the tips.

Table I gives data for the green weight, percentage moisture, percentage dry weight, and top-root ratios of the plants. The top-root ratios are obtained by dividing the green weight of the tops per 100 plants by the green weight of the roots per 100 plants. They are somewhat smaller than they should be because of the impossibility of entirely removing the surface water of the roots. It is evident that the sulphur deficient plants were smaller and that this difference became greater the longer the plants grew. Omitting sulphur from the nutrient solution affected the tops more than it did the roots. The top-root ratios are consistently greater for the plus sulphur plants. The tops of the plus sulphur plants were more succulent, as is evidenced by the higher moisture content of the leaves and stems. This difference was in general greater for the 1933 plants than for those grown in 1934. It is reversed in the roots, but probably has little significance, since it is difficult to make an accurate determination of the moisture content of roots.

## Effects of sulphur deficiency on chemical composition of plant

Nitrogen fractions.—Data for the chemical composition of the soy bean plants are given in tables II–VII. Only in the case of the 1934 plants are data recorded for the entire plant. The stem tips, petioles, etc., of the plants grown in 1933 were not analyzed. The percentages are figured on a dry weight basis. Since the plus sulphur plants have a lower percentage dry weight, this method of recording the data favors these plants in comparison with the minus sulphur plants; and in one or two instances reverses the difference which would exist if the data were calculated on a green weight basis. But percentage composition of roots figured on a green weight basis means little. In order, therefore, to have a uniform plan of recording the data for all parts of the plant and to save space, the data are recorded for the dry weight basis only.

Table II records data for the nitrogen fractions on a percentage

TABLE II

NITROGEN FRACTIONS

(PERCENTAGE, DRY WEIGHT BASIS)

| | Samples taken | | | | | |
|---|---|---|---|---|---|---|
| | April 22, 1933 | | May 5, 1933 | | April 21, 1934 | |
| | Plus S | Minus S | Plus S | Minus S | Plus S | Minus S |
| Total N | | | | | | |
| Leaves | 4.219 | 3.627 | 4.271 | 4.019 | 6.072 | 6.165 |
| Stems | 2.013 | 2.421 | 1.408 | 2.928 | 1.172 | 2.885 |
| Roots | 2.926 | 2.889 | 2.445 | 2.698 | 2.737 | 2.406 |
| Stem tips, etc. | | | | | 2.622 | 3.165 |
| Total organic N | | | | | | |
| Leaves | 4.127 | 3.435 | 4.218 | 3.874 | 5.999 | 5.934 |
| Stems | 1.705 | 2.154 | 1.226 | 2.736 | 1.058 | 2.658 |
| Roots | 2.448 | 2.474 | 2.126 | 2.195 | 2.598 | 2.160 |
| Stem tips, etc. | | | | | 2.420 | 2.949 |
| Soluble organic N | | | | | | |
| Leaves | 0.366 | 0.525 | 0.428 | 0.585 | 0.342 | 0.345 |
| Stems | 0.500 | 1.084 | 0.259 | 1.273 | 0.143 | 1.252 |
| Roots | 0.120 | 0.528 | 0.151 | 0.796 | 0.170 | 0.132 |
| Stem tips, etc. | | | | | 0.249 | 0.770 |
| Ammonia N | | | | | | |
| Leaves | 0.005 | 0.010 | 0.011 | 0.013 | 0.007 | 0.005 |
| Stems | 0.009 | 0.018 | 0.007 | 0.024 | 0.006 | 0.016 |
| Roots | 0.016 | 0.020 | 0.018 | 0.034 | 0.015 | 0.016 |
| Stem tips, etc. | | | | | 0.010 | 0.015 |
| Amino N | | | | | | |
| Leaves | 0.147 | 0.301 | 0.174 | 0.200 | 0.200 | 0.186 |
| Stems | 0.234 | 0.498 | 0.205 | 0.536 | 0.169 | 0.544 |
| Roots | 0.158 | 0.308 | 0.174 | 0.351 | 0.142 | 0.155 |
| Stem tips, etc. | | | | | 0.218 | 0.254 |
| Amide N | | | | | | |
| Leaves | 0.028 | 0.088 | 0.049 | 0.085 | 0.061 | 0.060 |
| Stems | 0.141 | 0.317 | 0.060 | 0.322 | 0.025 | 0.372 |
| Roots | 0.038 | 0.160 | 0.053 | 0.187 | 0.057 | 0.085 |
| Stem tips, etc. | | | | | 0.049 | 0.092 |
| Nitrate N | | | | | | |
| Leaves | 0.092 | 0.192 | 0.053 | 0.145 | 0.073 | 0.231 |
| Stems | 0.308 | 0.267 | 0.182 | 0.192 | 0.114 | 0.227 |
| Roots | 0.478 | 0.425 | 0.329 | 0.503 | 0.139 | 0.246 |
| Stem tips, etc. | | | | | 0.202 | 0.216 |

basis. There is an accumulation of ammonia nitrogen, amino nitrogen, amide nitrogen, and nitrate nitrogen in the minus sulphur as compared with the plus sulphur plants. The stems show this accumulation more than the roots, except in the case of nitrate nitrogen. The difference becomes greater the longer the plants grow. Owing largely to this great accumulation of the soluble organic nitrogen fractions, the percentage of soluble organic nitrogen, total organic nitrogen, and even total nitrogen is greater in the stems of the sulphur deficient plants than in those of the plus sulphur plants. This is not consistently true of the leaves and roots.

It should be mentioned that in certain cases the sum of the soluble nitrogen fractions, including the nitrates, is a little greater than the total nitrogen in the alcoholic extract (soluble organic nitrogen plus nitrates). This indicates some slight error in the analyses of these samples. WEBSTER (**36**) noted a decrease in amino nitrogen and an increase in ammonia nitrogen in alcoholic solution. STUART (**35**) reports difficulties in the determination by the Van Slyke method of amino nitrogen in the alcoholic extract of apple tissue, owing to the presence of some gas not absorbed by the potassium permanganate. But there was no evidence of a discrepancy such as WEBSTER reports. Ammonia nitrogen was always low in amount, and no difficulty such as STUART reports was experienced in the determination of amino nitrogen.

The facts that the discrepancy just mentioned occurs only in part of the samples, that when it occurs it is nearly always small, and that the analyses of plants at different stages of growth and of plants grown in different years give similar results, certainly indicate that the data of table II give rather closely the correct situation as to the percentage and distribution of the nitrogen fractions in the plus and minus plants.

Table III records the data for the nitrogen fractions on an absolute basis. Despite the greater growth of the plus sulphur plants, the totals for the sulphur deficient plants are greater for soluble organic nitrogen, ammonia nitrogen, amino nitrogen, amide nitrogen, and nitrate nitrogen than for the plus sulphur plants, except in the case of the ammonia determination of April 21, 1934, and the nitrate total of April 22, 1933. There is some variation in regard to these

TABLE III

NITROGEN FRACTIONS

(ABSOLUTE GRAMS PER 100 PLANTS)

| | Samples taken | | | | | |
|---|---|---|---|---|---|---|
| | April 22, 1933 | | May 5, 1933 | | April 21, 1934 | |
| | Plus S | Minus S | Plus S | Minus S | Plus S | Minus S |
| Total N | | | | | | |
| Leaves | 2.118 | 1.404 | 4.688 | 2.820 | 5.448 | 4.006 |
| Stems | 0.815 | 0.968 | 1.204 | 1.895 | 1.067 | 1.715 |
| Roots | 0.633 | 0.551 | 1.093 | 1.005 | 1.265 | 1.026 |
| Stem tips, etc. | | | | | 0.889 | 0.738 |
| Total | 3.566 | 2.923 | 6.985 | 5.720 | 8.669 | 7.485 |
| Total organic N | | | | | | |
| Leaves | 2.072 | 1.330 | 4.630 | 2.718 | 5.383 | 3.856 |
| Stems | 0.691 | 0.861 | 1.049 | 1.771 | 0.963 | 1.580 |
| Roots | 0.530 | 0.047 | 0.947 | 0.818 | 1.201 | 0.921 |
| Stem tips, etc. | | | | | 0.821 | 0.695 |
| Total | 3.293 | 2.238 | 6.626 | 5.307 | 8.368 | 7.052 |
| Soluble organic N | | | | | | |
| Leaves | 0.184 | 0.203 | 0.470 | 0.410 | 0.307 | 0.224 |
| Stems | 0.203 | 0.433 | 0.222 | 0.824 | 0.130 | 0.744 |
| Roots | 0.026 | 0.101 | 0.068 | 0.297 | 0.078 | 0.056 |
| Stem tips, etc. | | | | | 0.085 | 0.154 |
| Total | 0.413 | 0.737 | 0.760 | 1.528 | 0.600 | 1.178 |
| Ammonia N | | | | | | |
| Leaves | 0.002 | 0.004 | 0.013 | 0.009 | 0.007 | 0.003 |
| Stems | 0.003 | 0.007 | 0.006 | 0.015 | 0.005 | 0.009 |
| Roots | 0.003 | 0.003 | 0.008 | 0.013 | 0.007 | 0.007 |
| Stem tips, etc. | | | | | 0.004 | 0.003 |
| Total | 0.008 | 0.014 | 0.027 | 0.037 | 0.023 | 0.022 |
| Amino N | | | | | | |
| Leaves | 0.073 | 0.116 | 0.191 | 0.140 | 0.180 | 0.120 |
| Stems | 0.094 | 0.199 | 0.176 | 0.347 | 0.154 | 0.323 |
| Roots | 0.034 | 0.058 | 0.077 | 0.131 | 0.065 | 0.066 |
| Stem tips, etc. | | | | | 0.073 | 0.050 |
| Total | 0.201 | 0.373 | 0.444 | 0.618 | 0.472 | 0.559 |
| Amide N | | | | | | |
| Leaves | 0.014 | 0.034 | 0.054 | 0.060 | 0.054 | 0.039 |
| Stems | 0.057 | 0.127 | 0.052 | 0.208 | 0.022 | 0.221 |
| Roots | 0.008 | 0.030 | 0.024 | 0.069 | 0.026 | 0.036 |
| Stem tips, etc. | | | | | 0.016 | 0.018 |
| Total | 0.079 | 0.191 | 0.130 | 0.337 | 0.118 | 0.314 |
| Nitrate N | | | | | | |
| Leaves | 0.046 | 0.074 | 0.058 | 0.102 | 0.065 | 0.150 |
| Stems | 0.124 | 0.107 | 0.155 | 0.124 | 0.104 | 0.135 |
| Roots | 0.103 | 0.080 | 0.146 | 0.187 | 0.064 | 0.105 |
| Stem tips, etc. | | | | | 0.068 | 0.043 |
| Total | 0.273 | 0.261 | 0.359 | 0.413 | 0.301 | 0.433 |

fractions in different parts of the plant. As already stated, data were secured for the entire plant only for the analysis of April 22, 1934. But the totals for total organic nitrogen and total nitrogen are consistently greater for the plants grown in a complete nutrient solution, showing, as is to be expected, that the large plus sulphur plants synthesized a larger amount of nitrogenous material. Even here, however, the stems of the minus sulphur plants are higher, owing to their greater accumulation of the soluble organic nitrogen compounds.

Table IV records the data for the carbohydrate fractions on a percentage basis. The main points are that in general the sugars are higher in the plants grown in a complete nutrient solution but that starch and hemicellulose accumulate in the sulphur deficient plants. This difference in starch is greater in the stems and roots of the May 5, 1933, analysis than at the time of the earlier analysis, although the actual percentages are less in all parts of the plant except in the plus sulphur roots. Although the actual starch content of the plant is less later in the growth period, the hemicellulose percentage is greater, especially in minus sulphur leaves, and both the plus and minus sulphur stems. This may mean that as the plants get older the hemicellulose increases at the expense of the starch. Sulphur deficiency does not result in as great a piling up of hemicellulose as of starch; yet the minus sulphur leaves of the May 5, 1933, analysis have more than 2 per cent more hemicellulose than the plus sulphur leaves. The difference in favor of the high sulphur stems of the May 5 analysis is reversed if the percentages are calculated on the green weight basis. Adding the total sugars and starch together, it is found that in general the sulphur deficient plants of the two 1933 analyses have a higher percentage, but this is not true of the 1934 plants. When the percentage of total carbohydrates is figured, however, the low sulphur plants of all three analyses are higher, in most of their parts, although the differences are small in the 1934 plants. Total sugars plus starch and total carbohydrates were figured for the total weight of leaves, stems, and roots of the two 1933 samplings and for the entire plant of the 1934 series, with the results given in the last two horizontal columns of table V. It may be noted that with the carbohydrates figured in either one of these ways, the 1933

sulphur deficient plants of both analyses were high carbohydrate plants as compared with the plus sulphur plants; while in the case

TABLE IV

CARBOHYDRATE FRACTIONS

(PERCENTAGE, DRY WEIGHT)

| | Samples taken | | | | | |
|---|---|---|---|---|---|---|
| | April 22, 1933 | | May 5, 1933 | | April 21, 1934 | |
| | Plus S | Minus S | Plus S | Minus S | Plus S | Minus S |
| Total sugars | | | | | | |
| Leaves | 3.792 | 4.479 | 3.091 | 2.676 | 1.688 | 1.544 |
| Stems | 3.218 | 1.580 | 2.692 | 1.070 | 2.3084 | 0.862 |
| Roots | 3.084 | 2.588 | 3.412 | 2.461 | 1.822 | 1.315 |
| Stem tips, etc. | | | | | 1.603 | 0.926 |
| Reducing sugars | | | | | | |
| Leaves | 1.245 | 1.174 | 1.109 | 0.930 | 0.682 | 0.481 |
| Stems | 1.411 | 0.411 | 1.653 | 0.300 | 1.794 | 0.404 |
| Roots | 1.461 | 0.872 | 1.562 | 1.048 | 1.263 | 0.712 |
| Stem tips, etc. | | | | | 1.416 | 0.559 |
| Sucrose | | | | | | |
| Leaves | 2.419 | 3.139 | 1.883 | 1.659 | 0.955 | 1.012 |
| Stems | 1.717 | 1.110 | 0.986 | 0.732 | 0.487 | 0.440 |
| Roots | 1.343 | 1.630 | 1.757 | 1.341 | 0.530 | 0.572 |
| Stem tips, etc. | | | | | 0.179 | 0.349 |
| Starch | | | | | | |
| Leaves | 10.564 | 14.140 | 7.560 | 7.067 | 2.324 | 2.338 |
| Stems | 8.586 | 10.677 | 3.894 | 8.386 | 1.896 | 2.318 |
| Roots | 2.740 | 2.614 | 2.071 | 3.334 | 1.835 | 1.811 |
| Stem tips, etc. | | | | | 2.875 | 3.324 |
| Hemicelluloses | | | | | | |
| Leaves | 4.784 | 5.227 | 4.706 | 7.047 | 4.790 | 5.046 |
| Stems | 9.686 | 10.173 | 11.878 | 11.767 | 12.073 | 11.612 |
| Roots | 9.480 | 9.228 | 8.644 | 8.888 | 7.578 | 8.718 |
| Stem tips, etc. | | | | | 8.259 | 8.536 |
| Total sugars plus starch | | | | | | |
| Leaves | 14.356 | 18.619 | 10.651 | 9.743 | 4.012 | 3.882 |
| Stems | 11.804 | 12.257 | 6.586 | 9.457 | 4.204 | 3.180 |
| Roots | 5.824 | 5.202 | 5.483 | 6.586 | 3.657 | 3.126 |
| Stem tips, etc. | | | | | 4.478 | 4.250 |
| Total carbohydrates | | | | | | |
| Leaves | 19.140 | 23.846 | 15.357 | 16.890 | 8.802 | 8.928 |
| Stems | 21.490 | 22.430 | 18.464 | 21.224 | 16.277 | 14.792 |
| Roots | 15.304 | 14.430 | 14.127 | 15.474 | 11.235 | 11.844 |
| Stem tips, etc. | | | | | 12.737 | 12.786 |
| Total sugars plus starch | 11.976 | 13.402 | 8.240 | 8.780 | 4.076 | 3.505 |
| Total carbohydrates | 18.651 | 21.431 | 16.236 | 18.000 | 12.354 | 11.869 |

of the 1934 plants the difference is slightly in favor of plants grown in a complete nutrient solution.

In table V the carbohydrates are figured on an absolute basis. The totals for the sugars are higher for the plus sulphur plants, and in general this is true for the different parts of the plant. This is to be expected, since the high sulphur plants were larger and also had a larger percentage sugar content. Despite the higher percentage of starch and hemicellulose of the sulphur deficient plants, however, due to the larger amount of tissue formed by the plus sulphur plants, the totals for these substances are larger for the latter plants except in the case of starch of the April 22, 1933, analysis. This is not consistently true, however, of the different parts of the plant, except for starch of the April 21, 1934, analysis. The totals for total sugars plus starch and total carbohydrates are consistently larger for the high sulphur plants.

SULPHUR FRACTIONS.—Tables VI and VII record a few results for the sulphur fractions of the 1934 plants. The plants grown in a complete nutrient solution have a higher percentage of total sulphur and sulphate sulphur; but a greater proportion of the total sulphur of the sulphur deficient plants is present in the form of organic sulphur than is true of the plus sulphur plants. The minus sulphur leaves have a higher percentage of organic sulphur than the plus sulphur, while the stems of the two series are the same in this respect. There was very little organic sulphur or sulphate sulphur in the alcoholic extract. Nearly all of both fractions was found in the residue. On an absolute basis, the plants grown in a complete nutrient solution are higher in all three fractions.

MICROCHEMICAL DATA.—Microchemical tests were made for starch, reducing sugars, sulphates, and nitrates. The data do not add much to the macrochemical data recorded in the tables, but in general confirm them. One point of interest appeared in the microchemical tests for starch on the 1934 plants. The quantitative data show that there is a higher percentage of starch in the minus sulphur stems, although the difference is small. Microchemical tests indicated that near the base the plus sulphur stems were somewhat higher in starch but that near the tip the two series were about equal. In fact, tests on some of the plants indicated that the sulphur deficient

TABLE V

CARBOHYDRATE FRACTIONS

(ABSOLUTE GRAMS PER 100 PLANTS)

| | Samples taken | | | | | |
|---|---|---|---|---|---|---|
| | April 22, 1933 | | May 5, 1933 | | April 21, 1934 | |
| | Plus S | Minus S | Plus S | Minus S | Plus S | Minus S |
| Total sugars | | | | | | |
| Leaves | 1.904 | 1.733 | 3.393 | 1.878 | 1.514 | 1.003 |
| Stems | 1.303 | 0.632 | 2.303 | 0.693 | 2.102 | 0.513 |
| Roots | 0.667 | 0.492 | 1.520 | 0.917 | 0.842 | 0.561 |
| Stem tips, etc. | | | | | 0.549 | 0.185 |
| Total | 3.207 | 2.857 | 7.216 | 3.488 | 5.007 | 2.262 |
| Reducing sugars | | | | | | |
| Leaves | 0.625 | 0.454 | 1.217 | 0.652 | 0.611 | 0.312 |
| Stems | 0.571 | 0.164 | 1.415 | 0.194 | 1.634 | 0.240 |
| Roots | 0.316 | 0.165 | 0.695 | 0.390 | 0.584 | 0.304 |
| Stem tips, etc. | | | | | 0.480 | 0.111 |
| Total | 1.512 | 0.783 | 3.327 | 1.236 | 3.309 | 0.967 |
| Sucrose | | | | | | |
| Leaves | 1.214 | 1.215 | 2.066 | 1.164 | 0.857 | 0.657 |
| Stems | 0.695 | 0.444 | 0.844 | 0.474 | 0.444 | 0.261 |
| Roots | 0.290 | 0.310 | 0.782 | 0.500 | 0.245 | 0.244 |
| Stem tips, etc. | | | | | 0.060 | 0.069 |
| Total | 2.199 | 1.969 | 3.692 | 2.138 | 1.606 | 1.231 |
| Starch | | | | | | |
| Leaves | 5.305 | 5.474 | 8.298 | 4.959 | 2.085 | 1.519 |
| Stems | 3.476 | 4.270 | 3.332 | 5.429 | 1.726 | 1.378 |
| Roots | 0.593 | 0.497 | 0.922 | 1.242 | 0.848 | 0.772 |
| Stem tips, etc. | | | | | 0.975 | 0.665 |
| Total | 9.374 | 10.241 | 12.552 | 11.630 | 5.634 | 4.334 |
| Hemicelluloses | | | | | | |
| Leaves | 2.402 | 2.023 | 5.166 | 4.945 | 4.298 | 3.279 |
| Stems | 3.921 | 4.069 | 10.164 | 7.617 | 10.996 | 6.903 |
| Roots | 2.052 | 1.755 | 3.850 | 3.311 | 3.503 | 3.719 |
| Stem tips, etc. | | | | | 2.801 | 1.709 |
| Total | 8.375 | 7.847 | 19.180 | 15.873 | 21.598 | 15.610 |
| Total sugars plus starch | | | | | | |
| Leaves | 7.209 | 7.207 | 11.691 | 6.837 | 3.599 | 2.522 |
| Stems | 4.779 | 4.902 | 5.635 | 6.122 | 3.828 | 1.891 |
| Roots | 1.260 | 0.989 | 2.442 | 2.159 | 1.690 | 1.333 |
| Stem tips, etc. | | | | | 1.524 | 0.850 |
| Total | 13.248 | 13.098 | 19.768 | 15.118 | 10.641 | 6.596 |
| Total carbohydrates | | | | | | |
| Leaves | 9.611 | 9.230 | 16.857 | 11.782 | 7.897 | 5.801 |
| Stems | 8.700 | 8.971 | 15.799 | 13.739 | 14.824 | 8.794 |
| Roots | 3.312 | 2.744 | 6.292 | 5.470 | 5.193 | 5.052 |
| Stem tips, etc. | | | | | 4.328 | 2.559 |
| Total | 21.623 | 20.945 | 38.948 | 30.991 | 32.242 | 22.206 |

stems had a little higher percentage of starch near the tip than the plants grown in a complete nutrient solution. Since, when the entire stem is analyzed together the low sulphur stems have a higher percentage, it seems likely that some of the starch present in the lower part of sulphur deficient stems is hydrolyzed to sugar, and that the sugar is translocated to the tips of the plants, where part of it is changed back to starch. It is probable that, accompanying this

TABLE VI

SULPHUR FRACTIONS

(PERCENTAGE, DRY WEIGHT)

| | Total S | | Sulphate S | | Organic S | |
|---|---|---|---|---|---|---|
| | Leaves | Stems | Leaves | Stems | Leaves | Stems |
| Plus S | 0.430 | 0.270 | 0.223 | 0.223 | 0.207 | 0.047 |
| Minus S | 0.342 | 0.123 | 0.106 | 0.076 | 0.236 | 0.047 |

TABLE VII

SULPHUR FRACTIONS

(ABSOLUTE GRAMS PER 100 PLANTS)

| | Total S | | Sulphate S | | Organic S | |
|---|---|---|---|---|---|---|
| | Leaves | Stems | Leaves | Stems | Leaves | Stems |
| Plus S | 0.386 | 0.246 | 0.200 | 0.203 | 0.186 | 0.043 |
| Minus S | 0.222 | 0.073 | 0.069 | 0.045 | 0.153 | 0.028 |

starch transfer, some of the chloroplasts in the lower part of the minus sulphur stems break down, the proteins being hydrolyzed and the material translocated to the tips of the plants, where it is used in stem elongation, as will be discussed later.

Boron injury.—The question naturally arises whether boron injury may not in part account for the differences between the plus sulphur and minus sulphur plants of 1933. While it is possible that this may be true, it is not thought that the results obtained are much affected by this factor. In the first place, although exact counts were not made, boron deficiency seemed to affect the plants of the two

series about equally. Again, although the April 22, 1933, analysis was made before there was much recovery from boron injury and included some plants with dead tips, at the time of the May 5, 1933, analysis most of the plants had recovered and had put out long side shoots. All the plants sampled at this time had well developed side shoots. Yet the two analyses show similar differences between the plus and minus sulphur plants except that they are often greater in the case of the latter analysis, as would be expected. Finally, no boron injury developed in the 1934 plants; yet the differences in the chemical composition of the two series are similar to those of the 1933 plants, although decidedly smaller in the case of starch and hemicellulose.

SULPHUR DEFICIENCY IN OTHER PLANTS.—Several other plants were grown in 1934 to compare with the soy bean. These were sunflower, rape, kale, and mustard. The symptoms of sulphur deficiency were about the same as in the soy bean. The minus sulphur plants of each series were smaller, shorter, and not so green as the plus sulphur plants. The effects of sulphur deficiency on stems and leaves were very pronounced in the sunflower, the leaves being smaller and the stems thinner. While the mustard plainly showed the effects of lack of sulphur, it was more in the size of the plants than in color. This is perhaps not strange, since so much of the sulphur is present in mustard oil rather than in protein. The higher mustard oil content of the plus sulphur mustard leaves was very evident to the taste, which was much more acrid than in the case of the minus sulphur leaves. This difference was not so marked in the case of the other Cruciferae, kale, and rape.

No analyses were made of any of these plants. Table VIII gives the green weights and the top-root ratios. The top-root ratios are figured on the green weight basis and are smaller than they should be because of the excess surface water on the roots. It was impossible to remove all of this with filter paper. No analyses were made. It is planned later to grow these plants in quantity and make analyses to see how they compare in chemical composition with the soy bean.

## Discussion

COLOR, SIZE OF LEAVES, THICKNESS OF STEMS.—The main symptoms of sulphur deficiency in the soy bean were the yellow-green

color of the leaves, the smaller size of the leaves, and the thinner stems as compared with the plants grown in a complete nutrient solution.

The effects of sulphur deficiency on the size and color of the plant have been known for a long time. Even during the early history of our government sulphur in the form of gypsum was in common use, and resulted in increased yield. The darker green color of the plants was also noticed. CROCKER (**4**) reviews some of this early literature. More recently in the northwest the effects of sulphur fertilizers in producing larger, darker green plants has been noted in the case of alfalfa (**26, 32**). Great increases in yield have been obtained and it

TABLE VIII

GREEN WEIGHT PER 25 PLANTS, TOP-ROOT RATIO

| | SUNFLOWER | | KALE | | RAPE | | WHITE MUSTARD | |
|---|---|---|---|---|---|---|---|---|
| | HIGH S | LOW S | HIGH S | LOW S | HIGH S | LOW S | HIGH S | LOW S |
| Tops | 780.0 | 430.2 | 201.6 | 43.4 | 351.2 | 223.8 | 155.4 | 104.3 |
| Roots | 130.0 | 109.3 | 35.4 | 8.2 | 52.9 | 38.0 | 54.4 | 47.6 |
| Entire plant | 910.0 | 539.5 | 237.0 | 51.6 | 404.1 | 311.8 | 209.8 | 151.9 |
| Top-root ratio | 6.000 | 3.933 | 5.690 | 5.260 | 6.631 | 5.874 | 2.856 | 2.188 |

has been definitely proved that the effect is due to the sulphur component of the fertilizer used.

Until rather recently most of the work with sulphur fertilizers has been done in the field, or in the greenhouse with soil cultures. Plants belonging to either the Leguminosae or the Cruciferae have given the best response. This is naturally true since in general the plants of these two families are highest in sulphur owing to the high content of the legumes in protein which contains sulphur and of the crucifers in mustard oils and other sulphur containing compounds. But using sand cultures, DULEY (**6**) noted the effects of sulphur deficiency in producing smaller, less green corn plants. And recently NIGHTINGALE (**22**) in the tomato, and STOREY and LEACH (**34**) in the tea bush secured symptoms of sulphur deficiency that correspond closely to those obtained in the soy bean.

The gradation of greenness in regard to position of the leaves on

the plant seems to vary with different plants. While it was not perhaps absolutely regular, the upper leaves of the soy bean generally became yellow first. There was an increase in greenness from the top to the bottom of the plant (fig. 2). Sometimes the lower leaves also became yellow, but since many of the lower leaves died and fell off, the yellow color was probably due to the decomposition of the chlorophyll which accompanied the general breakdown of the cell contents as death approached. This gradation of greenness due to sulphur deficiency seems to correspond with the results of STOREY and LEACH (**34**) for the tea bush, but is in contrast to the results of NIGHTINGALE (**22**) for the tomato. In the case of the tomato the lower leaves became yellow first.

As NIGHTINGALE points out (**22**), the symptoms of sulphur deficiency are similar to the deficiency of other elements, although the gradation of greenness varies. A nitrogen deficient plant is yellow and has small leaves and stems (**15**). And at certain stages, at least, the symptoms of lack of potassium (**24**), phosphorus (**12**), or calcium (**25**) are similar to those of sulphur. That the effects of a deficiency of each of these elements should be similar, especially in regard to color, is perhaps not strange, for ECKERSON (**11**) has shown that when these elements are lacking in the nutrient solution the tissues of the resulting plants have a low reducase activity. So the use of nitrates in the synthetic processes of the plant is interfered with and nitrogen is necessary for the synthesis of chlorophyll and the proteins of the chloroplast. In the case of calcium there is also poor absorption of nitrates (**25**). Thus it would seem that these elements are having their effects indirectly by interfering with the assimilation of nitrates. NIGHTINGALE (**22**) states: "The minus-sulphur [tomato] plants looked as if they had been gradually but not completely limited as to their nitrogen supply." This was true also of the soy bean. While the plants were chlorotic, this condition was not so pronounced as in advanced stages of nitrogen deficiency. And ECKERSON (**11**) found that although sulphur deficiency caused a lower amount of reducase in the tomato, there was still enough present to allow the reduction of nitrates to go on slowly. Sulphur deficiency does not cause as great a reduction in reducase as deficiency of potassium, phosphorus, or calcium.

Although the chlorotic condition of minus sulphur plants would

seem to be due mainly to poor nitrate assimilation, two other points should be mentioned. One is that although sulphur is not needed as a building stone for the synthesis of chlorophyll, it is needed for the synthesis of most if not all plant proteins. So it is probably needed for the synthesis of the proteins of the chloroplast and its absence might result in the development of fewer chloroplasts, and thus affect the degree of greenness to a certain extent. Also, the synthesis of chlorophyll is one of the life processes of the plant, and the deficiency of any element necessary for the healthy state and proper functioning of the plant may no doubt affect chlorophyll development.

STEM ELONGATION.—NIGHTINGALE (**22**) found that the minus sulphur tomato plants were as tall as, and in some cases taller than, the plants grown in a complete nutrient solution, but they were much smaller in diameter. Much the same situation is found in the soy bean. The stems of both plus and minus sulphur plants elongated remarkably and assumed the twining habit (fig. 1). Of course the habit of neither set of plants was normal, because of the use of electric lights. Stem elongation has often been noticed, when electric lights have been used or when certain parts of the spectrum have been eliminated (**8, 29, 38**). But the point is that leaving sulphur out of the nutrient solution did not prevent stem elongation. The sulphur deficient plants, although not so tall on the average as the plants grown in a complete nutrient solution, still showed decided stem elongation. Some of them were about as tall as the plus sulphur plants, but were very small in diameter, in some cases being less than 2 mm. in diameter at the tips.

This is a rather unique situation. Deficiency of an essential element usually causes the stunting of the plant, which is shown not only in smaller plant parts but in shorter stems. Deficiency of sulphur results in less production of tissue, the plant parts are smaller (table I), but the stem may be as long as those of plants grown in a complete nutrient solution. Only in special situations does this seem to be true of other elements, as, for example, when plants deficient in nitrogen (**23**), potassium (**24**), or calcium (**25**) are placed in darkness. Then there is marked stem elongation. This subject will be referred to again in connection with a discussion of the effects of sulphur deficiency on the organic nitrogen fractions.

ROOT DEVELOPMENT.—Minus sulphur soy bean plants had poor root development, but the roots were not stunted as much as the tops, as shown by the top-root ratios (table I); in fact in the 1934 plants the roots of the sulphur deficient plants weighed almost as much as the plants grown in a complete nutrient solution.

This effect of sulphur deficiency in causing poor root development has been known for a considerable time, although the data given do not always permit a comparison of the effects on tops and roots. REIMER and TARTAR (**32**) emphasize the increased root development of alfalfa due to sulphur fertilization. Recently NIGHTINGALE (**22**) found that the roots of sulphur deficient tomato plants were of small diameter because there was not much cambial development and therefore very little secondary thickening. STOREY and LEACH (**34**) state that the roots of the tea bush suffering from yellows (which they show is a sulphur deficiency disease) may be poorly developed.

It is interesting to compare the effects of sulphur deficiency on root development with the effects of the deficiency of other elements. NIGHTINGALE (**22**) states that the roots of minus sulphur plants are similar to the roots of low nitrogen plants, and he has shown (**23**) that plus nitrate solutions cause greater growth of tops than of roots; or to state it conversely, nitrate deficiency stunts the tops more than it does the roots. This is similar to the effects of sulphur deficiency on the soy bean. HARRISON (**13**) found that nitrates increased the growth of the tops of golf grasses but not of the roots. WELTON (**37**) found that the roots of soy bean weighed 6.2 per cent as much as the tops when the plants were grown in sand, but only 3.4 per cent as much when grown in manure. RUSSELL (**33**) emphasizes the influence of phosphorus on root development. He states that the root systems of phosphate deficient cereals are stunted and more so than the tops, and that the addition of phosphates to such plants causes greater increase in tops than roots. But superphosphate was apparently used in these experiments, and as CROCKER (**5**) points out, the effect may have been due partly to the sulphates present. ECKERSON (**12**) studied the effects of phosphate deficiency without this complication. Her pictures show a decided stunting of both roots and tops. No weights are given.

## Effects of sulphur deficiency on chemical composition of plant

Accumulation of carbohydrates and nitrates.—Nitrates and carbohydrates may accumulate in plants as a result of several conditions. Nitrogen starved plants are high in carbohydrates (**15, 23**). This condition can be brought about either by withholding nitrates from the nutrient solution, in which case of course nitrates will be low or absent in the plant, or with nitrates in the nutrient solution, subjecting the plant to some condition which restricts nitrate reduction and thus the synthesis of organic nitrogenous compounds for which both nitrates and carbohydrates are necessary. In the latter case both nitrates and carbohydrates accumulate. Eckerson (**11, 12**) has shown that tomato plants deficient in potassium or phosphorus are low in reducase. She found that the minus phosphorus tomato plants were high in carbohydrates and nitrates, and Nightingale (**24**) has shown that the same thing may be true of potassium deficient tomato plants. Carbohydrates accumulate in calcium deficient tomato plants (**25**), but here, although the reducase content is low, absorption of nitrates is poor and this alone would no doubt account for the accumulation of carbohydrates. Because of poor absorption, nitrates are not high in amount.

Eckerson (**11**) found that sulphur deficient tomato plants are also low in reducase. Nightingale (**22**) found that nitrates and carbohydrates accumulate in these plants, and he states that this is due to the poor reduction of nitrates and synthesis of amino acids and various other organic nitrogenous compounds. A similar situation exists in the soy bean and it is no doubt to be explained on the same basis. The minus sulphur soy bean plants are high in nitrates. But in regard to the carbohydrates the situation is not so clear as in the tomato. Reducing sugars, sucrose, and starch accumulate in the sulphur deficient tomato plants. Hemicelluloses were not determined. Starch and hemicellulose are high in the minus sulphur soy bean plants, but the sugars are rather uniformly higher in the plus sulphur plants (table IV). Also, when total sugars plus starch or total carbohydrates are figured for the entire plant, the 1933 plants are high carbohydrate plants, and in general the same thing is true for the different parts of the plant. So the preceding explanation of

carbohydrate accumulation might be regarded as true for these plants. But when the carbohydrates are figured in these ways for the entire plant of the 1934 experiment, the plus sulphur plants are somewhat higher. And in the case of the different parts of the plant of the 1934 series, although total carbohydrates are a little higher in most cases in the minus sulphur plants, the reverse is true of total sugars plus starch. These plants, therefore, do not show the accumulation of carbohydrates as much as those of 1933. For the reasons just outlined, the effect of sulphur deficiency on carbohydrate accumulation is not so clear in the soy bean as in the tomato. But it would seem that the deficiency of this element may in some plants cause the accumulation of certain forms of carbohydrates rather than of all carbohydrates, for the plants of both years are uniform in regard to the higher percentages of starch and hemicellulose of the minus sulphur plants.

Table IV shows that the differences between the sulphur deficient plants and those grown in a complete nutrient solution are smaller for starch and hemicellulose for the 1934 plants than for those grown in 1933. It may be that variations in temperature and light conditions of the two years account for this, and of course these factors may also be of importance in accounting for the differences noted for relative accumulation of carbohydrates in the plants of the two years when figured as total sugars plus starch or total carbohydrates. For the reasons already noted, it is not thought that the boron deficiency which developed in the 1933 plants accounts for the differences.

It is not known in what degree hemicelluloses serve as reserve food materials of the plant. NIGHTINGALE (**21**) considers that hemicelluloses are not so important in the plant's metabolism as are starch and dextrin. CLEMENTS (**3**), however, considers that the hemicelluloses may be rather important metabolically. There is perhaps a little support in the data of table IV for the idea that the hemicelluloses may serve as temporary food reserves and therefore be important in the metabolism of the plant. At least the differences between the plus and minus plants in hemicellulose content is similar to, although not so great as (especially in the 1933 plants), the differences in starch.

ORGANIC NITROGEN FRACTIONS.—NIGHTINGALE (**22**) finds that

minus sulphur tomato plants are high in the soluble organic nitrogenous fractions. This is also true of the soy bean, especially in the stems (table II). This situation has been found to occur in plants as a result of various conditions. For example, a plant that is actively vegetative owing to an abundance of nitrates is high in these organic forms of soluble nitrogen, as compared with the nitrogen deficient plant (**23**). Here the organic nitrogenous compounds are being synthesized more rapidly than they can be combined to form protein. If plants deficient respectively in nitrogen (**23**) or potassium (**24**) are put in continuous darkness, there is stem elongation; and accompanying this, amide nitrogen, amino nitrogen, etc., increase in percentage and carbohydrates decrease. Here proteolysis occurs. Proteins are being hydrolyzed and reutilized in stem elongation. Sometimes the deficiency of an essential element results in the piling up of these soluble organic nitrogenous compounds even when the plants are in the light. NIGHTINGALE (**24**) found this true of potassium deficient tomato plants at a late stage, and it has also been found to be true of phosphorus deficient tomato plants (**16, 17**). NIGHTINGALE (**22**) attributes this situation in minus potassium or minus phosphorus tomato plants to proteolysis, which occurs shortly before the death of the plant and is accompanied by rather rapid stem elongation.

Sulphur deficient tomato plants are low in reducase (**11**). This no doubt is also true of the soy bean. Therefore the reduction of nitrates and the synthesis of proteins is much restricted. So the accumulation of these organic forms of soluble nitrogen could not be due to their rapid synthesis such as occurs in a highly vegetative plant supplied with an abundance of nitrates. The accumulation occurred while the plants were exposed to the full light period; it was not a case of proteolysis in darkness. There was no evidence that the minus sulphur plants were about to die. In fact, some of the sulphur deficient plants of 1933 were allowed to grow more than two weeks after the last samples were taken and they were still growing vigorously when the experiment was discontinued. Proteolysis which sometimes occurs shortly before the death of the plant does not seem to account for the piling up of these compounds. It seems to be due in the soy bean, as in the tomato (**22**), to a special type of proteolysis.

Both kinds of plants are able, under normal light conditions and while they are still healthy, to break down proteins, translocate the nitrogenous material, and reutilize it in stem elongation and leaf development. It is this power of the plants which accounts for the stem elongation already mentioned.

As has been pointed out in other cases of proteolysis (**22**), there is a decrease in the percentage of carbohydrates. But the sulphur deficient tomato plants were at all times high in reducing sugars, sucrose, and starch; yet they were actively breaking down proteins while these carbohydrates were accumulating. It is this fact which makes this a special case of proteolysis. The carbohydrate accumulation situation in the soy bean, however, seems to be somewhat different from that in the tomato. While the minus sulphur soy bean plants were higher in starch and hemicelluloses than the plus sulphur plants, they were decidedly lower in sugars, and the difference became greater in the later stages (table IV), indicating that the more directly available carbohydrates are lower in the sulphur deficient plants which are carrying on proteolysis. Also, although total sugars plus starch and total carbohydrates figured for the entire plant are higher in the minus sulphur plants of 1933, the reverse is true of the 1934 plants. So the carbohydrate situation in the soy bean might perhaps be considered to be more in line with that usually accompanying proteolysis than is true of the tomato.

There must have been, however, considerable synthesis of nitrogenous compounds by the minus sulphur plants, especially during the early part of the growth period, before reducase became deficient. A consideration of the data on an absolute basis becomes interesting in this connection (table III). The data are figured as grams per 100 plants. On April 22, 1933, the leaves, stems, and roots of the plus sulphur plants contained 32.93 mg. per plant of total organic nitrogen; the minus sulphur plants, 22.38 mg. This had increased by May 5 to 66.26 and 53.07 respectively. It is unfortunate that the stem tip, petioles, etc., were not analyzed. This was done in 1934, giving a total for the plus sulphur plant of 83.68 mg. per plant and the minus sulphur plant of 70.52 mg. The soy beans weighed on the average 167 mg. each. The seeds were not analyzed, but HENRY and MORRISON (**14**), in their compilation of the analyses of feeding ma-

terials, give 36.5 per cent as the average crude protein content of soy bean seeds (average of 121 analyses). Each seed then contains 60.9 mg. of crude protein, or, dividing by the usual factor, 6.25, 9.7 mg. of N. Making due allowance for differences in the protein content of different varieties and samples of soy bean seed, and regarding all of this protein as being hydrolyzed and translocated to the developing plant (which of course is no doubt untrue), it is seen that there must have been considerable synthesis of nitrogenous material by the sulphur deficient plants, and that this was going on more or less actively late in the growth period (compare data for April 22 and May 5). As already mentioned, ECKERSON found that sulphur deficiency does not result in as great a reduction of reducase in the tomato as the deficiency of other elements (11). There is enough reducase present to allow reduction of nitrates to go on slowly. With sulphates omitted from the nutrient solution, amino acids could not be combined to form proteins, since most, probably all, plant proteins contain sulphur. Therefore this newly synthesized nitrogenous material would accumulate in various soluble forms of nitrogen, and might account in part at least for the accumulation of the soluble forms of nitrogen in the minus sulphur plants (table II). With no sulphates in the nutrient solution it would not be expected that there would be present much cysteine, glutothione, or other sulphur containing nitrogenous compounds; and NIGHTINGALE (22) found these compounds largely absent from the organic sulphur of sulphur deficient tomato plants.

On the other hand, even in the minus sulphur plants some of the newly synthesized nitrogenous material might be proteinaceous in nature. The present method did not permit the determination of protein nitrogen, but the data of table III show that most of the organic nitrogenous material of both plus and minus sulphur plants was insoluble in 80 per cent alcohol. No sulphate was added intentionally to the nutrient solution applied to the minus sulphur plants, but there was some present as impurities in the salts used, and some sulphate present in the air might have dissolved in the moist sand. Previous work has shown that the atmosphere of Chicago contains considerable sulphur which is present mainly in the sulphate form (9). So a small part, at least, of the organic forms of soluble nitrogen

which table II shows accumulate in the sulphur deficient plants might represent a step on the way to protein. And of course the newly synthesized protein could be broken down proteolytically as already described.

The preceding considerations indicate that we should not depend entirely on proteolysis to account for the accumulation of the soluble organic nitrogenous fractions in the minus sulphur plants, and may have a bearing in explaining why the carbohydrate situation is different from that usually found in other cases of proteolysis.

CHEMICAL COMPOSITION AND CELL WALL THICKNESS.—The various conditions in plants which may lead to carbohydrate accumulation have already been discussed. It has been noticed repeatedly that accompanying this accumulation the cell walls of the cells in the cortical, phloem, and xylem regions of the stem are thicker than in the case of plants of lower carbohydrate content. NIGHTINGALE (**22**) found that the minus sulphur tomato plants, which were higher in carbohydrates than the plus sulphur plants, also had thicker cell walls in all parts of the stem. Not much attention was given to the anatomy of the soy bean plants of these experiments, but it was noticed that the stems of the sulphur deficient plants in which starch and hemicellulose accumulated were harder than those of the plus sulphur plants. This was noticed especially in the 1933 plants, and there was greater accumulation of starch and hemicellulose in the plants of this year than in those of 1934 (table IV). Perhaps starch and hemicellulose are more closely related to cell wall thickness than are the sugars, for the sugars were higher in the plus sulphur soy bean plants.

## Summary

1. The effects of sulphur deficiency were studied in the soy bean. It took considerable time for symptoms of sulphur deficiency to become apparent, but they were beginning to show in four weeks' time and had become pronounced in six weeks.

2. The main symptoms were the yellow-green color of the leaves, the smaller leaflets, and the thinner stems. These symptoms are similar to those that have been noted for other elements, for example, ni-

trogen, potassium, and phosphorus. This no doubt indicates a similar cause, namely, poor nitrate assimilation.

3. The upper leaves become yellow first. This is similar to the effect of sulphur deficiency in the tea bush and to calcium deficiency in the tomato, but is in contrast to deficiency of nitrogen, potassium, or sulphur in the latter plant. In the case of the latter elements, the lower leaves become yellow first.

4. Stem elongation was a prominent feature of both plus and minus sulphur plants because of the use of electric lights for part of the light period. Omitting sulphur from the nutrient solution caused some reduction in the height of the plants, but still the stems elongated remarkably. This is in contrast to the deficiency of other elements and is no doubt correlated with the ability of these plants to break down and reutilize proteins.

5. Sulphur deficiency affected the tops of the plants more than the roots. This is similar to the effects of nitrogen deficiency and also to some of the results reported for phosphorus deficient plants.

6. The stems of the minus sulphur plants were harder than those of the plus sulphur plants. Cell wall thickness has been found correlated with an accumulation of carbohydrates. In the case of the soy bean, it seems more correlated with the accumulation of starch and hemicelluloses than with all forms of carbohydrates.

7. Characteristic symptoms of boron deficiency appeared in a number of the plants grown in 1933. The numbers of plus sulphur and minus sulphur plants showing this injury were about equal. The addition of boron caused the quick formation of side shoots. It is not thought that boron deficiency affected the differences in the plus and minus sulphur plants.

8. The plus sulphur plants were more succulent than the sulphur deficient plants. In this they were like plants grown with plenty of nitrates in the nutrient solution.

9. Nitrates accumulated in the minus sulphur plants. This was because of poor nitrate assimilation. Deficiency of other elements may also cause this. It would be expected that in such circumstances carbohydrates would also accumulate. Starch and hemicelluloses were higher in the sulphur deficient plants, but sugars accumulated

in the plus sulphur plants. Sulphur deficiency apparently affects the form of carbohydrate accumulation in the soy bean. This is in contrast to the tomato, in which all forms of carbohydrates accumulate in the minus sulphur plants.

10. There is some evidence that hemicelluloses serve as reserve food materials in the soy bean.

11. The soluble organic nitrogenous fractions were higher in the minus sulphur plants, especially in the stems. This was at least partly due to proteolysis, and is important in accounting for the stem elongation of the minus sulphur plants. When proteolysis occurs, there is usually also a decrease in carbohydrates. The sulphur deficient plants were higher in starch and hemicelluloses but lower in sugars; therefore the proteolysis which occurs is of a special type. But since the minus sulphur plants are lower in sugars, these plants may perhaps be regarded as more in accord with the usual situation accompanying proteolysis than sulphur deficient tomato plants, in which all forms of carbohydrates accumulate.

12. Accumulation of the soluble organic nitrogenous fractions in the minus sulphur plants no doubt was partly due to the new synthesis of these compounds and the failure of the completion of protein synthesis because of the lack of sulphates, for nitrate reduction may no doubt go on slowly in sulphur deficient plants. This may have a bearing in explaining the somewhat abnormal situation in regard to the accumulation of carbohydrates during proteolysis.

13. Similar symptoms of sulphur deficiency were noted in the sunflower, kale, rape, and mustard. The influence of sulphur deficiency in reducing the mustard oil content was definitely evident in the mustard. The leaves of the minus sulphur plants were not nearly so acrid in taste as those of the plus sulphur plants.

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# MORPHOLOGY AND PHYSIOLOGY OF THE POME LENTICELS OF PYRUS MALUS[1]

HARRY F. CLEMENTS

(WITH NINE FIGURES)

## Introduction

The term lenticel, as established by DE CANDOLLE, applies to structures of the stem which typically appear as masses of tissue of the periderm which have enlarged to the point of rupturing the epidermis (3). Its function is regarded as concerned with gas exchange. Were this concept of a lenticel adhered to, the structures on the pome of the apple could scarcely be regarded as such. There are developed no such masses of periderm tissue; in fact it is seldom that periderm activity is associated with these structures. Fruit spots or dots might be more appropriate as descriptive terms were it not that they have been used to denote anything from diseases caused by fungi to insect punctures and even physiological breakdown. Hence, so far as this paper is concerned, the term lenticel will indicate the white, green, or yellowish brown spots common to all apple fruits; for although it does not carry the same connotation with reference to the pome as it does in woody stems, it describes the nature of the pome structures more nearly than do spots, dots, or stomata.

The structure and function of the lenticels of the mature pome have received little attention from investigators. It has been assumed, apparently, that they are similar to the lenticels of woody stems. Because of the possible importance of these structures in gas exchange, and because of their demonstrated importance as infection courts (1) for bluemold and other decay organisms, it was considered worth while to investigate their nature and function.

[1] Contribution no. 41 from the Botany Department of the State College of Washington.

## Historical review

ZSCHOKKE (**9, 10**) studied the morphology of the apple epidermis including the stomata. He reports that the stomata are completely formed by the time the pome is four weeks old and that at this time they are more or less uniformly distributed over the surface. As the apple increases in size most at the stem end and least at the calyx end, the stomata are distributed by the stretching of the epidermis. In the very young pome from two to ten stomata may be found per square millimeter of surface. ZSCHOKKE (**9, 10**), BROOKS (**2**), and KIDD and BEAUMONT (**5**) were unable to find stomata on the mature fruit and hence concluded that they took no part in gas exchange except in so far as they might act as lenticels. TETLEY (**7, 8**) gives an excellent account of the development of the stomata and treats of their change to lenticels, suggesting that their formation results from the breaking of stomata caused by the stretching of the epidermis. HAYLETT (**4**) reports that a lenticel results from a stoma whose substomatal cells become suberized. ZSCHOKKE found that lenticels may result from broken stomata or from scars caused by the falling out of trichomes.

The function of lenticels has generally been assumed to be that of gas exchange. MAGNESS and DIEHL (**6**) coated apples with paraffin and noted a decrease in the rate of carbon dioxide release and an accumulation of this gas in the intercellular spaces. The opinion that the cuticle is of some consequence in the exchange of gases was expressed by HAYLETT, who further states that the cavity in the apple opening at the calyx end is not an important passageway for gases.

## Experimentation

The structure of the lenticel was studied from prepared sections of apple skin cut at right angles to the arc connecting the stem and calyx ends of the apple, as well as sections cut parallel to it. The fruit was taken from local orchards as well as from orchards in the Yakima and Wenatchee valleys, and Prosser, in central Washington.[2] Open and closed lenticels were selected from the apples follow-

[2] The writer is indebted to Dr. KENNETH BAKER who collected the material in the central Washington orchards and who was of constant assistance throughout the work, and to the Department of Plant Pathology for the actual cost of shipment and storage.

ing submergence in a solution of methylene blue, The apples were allowed to remain in the solution for 24 hours, during which time the temperature in the room was allowed to drop some 10° C., causing a mild contraction of the fruit. In this way the dye solution was drawn into the apple slowly and without the possibility of rupturing the lenticels. Lenticels which showed a distinct halo of dye in the parenchymatous tissue below were regarded as open lenticels; those which remained uncolored were regarded as closed; and those which showed a surface adsorption of the dye but without its diffusion into the tissue beneath were at first called partly closed lenticels. These were later found to have the substomatal tissue thoroughly suberized and impermeable to water and dye, and hence were closed lenticels.

The lenticels so selected were fixed in Turtox, imbedded in paraffin, and sectioned. Some of the sections were stained with safranin and gentian violet, although the more satisfactory stain was Sudan III, which stained the cuticle heavily, the suberized tissue lightly, and the unmodified cellulose walls not at all. The sections were mounted in glycerin. Sections were made of lenticels taken from apples of the following horticultural varieties: Jonathan, Winesap, Delicious, Red Delicious, Golden Delicious, McIntosh, Pearmain, Winter Banana, Arkansas Black, Black Stayman, Stayman Winesap, Rome Beauty, Yellow Newtown, Spitzenburger, Grimes Golden, Black Twig, and Starking Delicious.

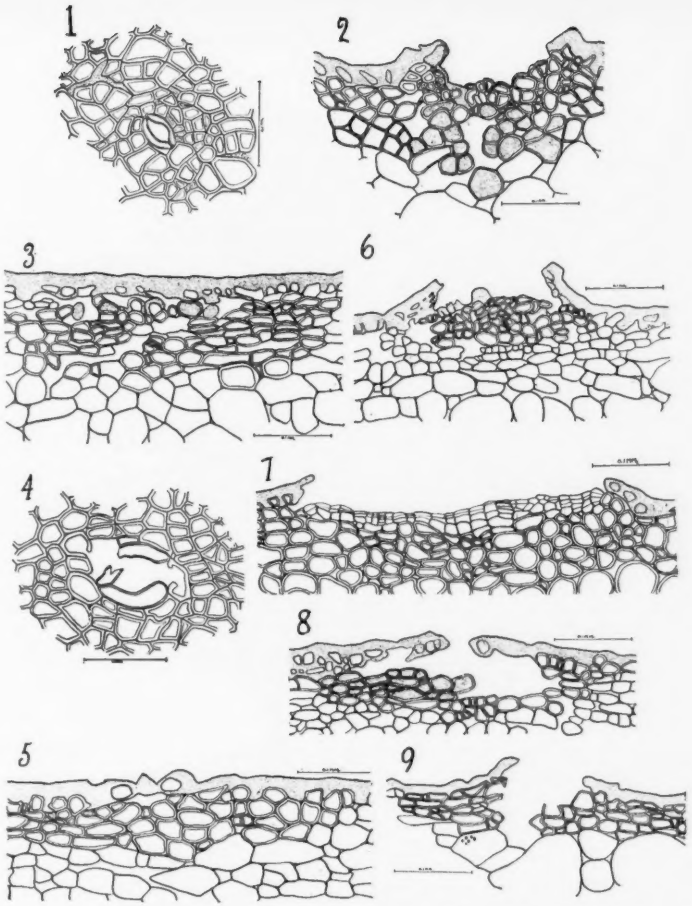
## Morphology of lenticel

Pome lenticels are derived from a variety of sources: from stomata which cease functioning as stomata early in the development of the pome; from breaks in the continuity of the epidermis caused by a complete removal of the trichomes characteristically associated with the young apple; and from other epidermal breaks brought about by the inability of the epidermis to keep pace with the expanding inner tissues. These last breaks are usually preceded by a cracking of the cuticle which definitely follows cell boundaries. This is as far as the break goes in many cases and a layer of cutin is formed and no lenticel develops.

Although in most cases the opening into the lenticel is a stoma

(fig. 1), the appearance of the lenticel is the result of change in the subepidermal cells. When the apple is still green and from two to four weeks old, even before the trichomes have dropped, the layers of cells immediately beneath the stomatal aperture become impregnated with a substance that appears to be tannin. This material seems to arise in the cells in which it is found. The cells become so modified that they remain a yellowish brown color while the apple enlarges and develops the red and green colors characteristic of the other hypodermal cells. Thus arises the lenticel or spotty appearance of the apple. That these cells are definitely changed is further indicated by the fact that they do not continue division and growth. In the mature apple, the cells immediately below the stoma appear as though radiating from the substomatal cavity. This evidently is the result of a considerable tension developed by the enlarging fruit; and as these subepidermal cells fail to relieve this tension by the multiplication of cells, they are pulled in such a way as to give the appearance of radiating from the lenticel. That there is much sliding of the subepidermal layers is indicated by the large numbers of intercellular spaces found between these layers in the mature apple (figs. 2, 3). In some instances the pull is sufficiently great to cause a rupture at the point of the stoma (fig. 4). The stoma otherwise remains intact and can be seen with its guard cells in the mature apple, especially if seen in surface view. It is difficult to understand the statements appearing in the literature that the stomata disappear. By far the greater proportion of the lenticels of the apple have uninjured but apparently inactive stomatal apparatus associated with them (fig. 1). In some apples, especially the Grimes Golden, the lenticel is associated with an opening caused by the complete removal of a trichome. This opening is not filled in by the adjacent cells and serves in a way comparable to that of an open stoma. This condition is not common, however. A crack in the epidermis causes the same type of modification of the hypodermal cells as was observed beneath a stoma (fig. 1).

As apples mature the guard cells of the stoma remain permanently open, probably as a result of the stretching which accompanies growth. Thus at first, when the stoma is no longer functional, the developing lenticel is open to the inward as well as to the outward




FIGS. 1–9.—Fig. 1, surface view of epidermis over lenticel from Jonathan apple but guard cells are unbroken; a crack in epidermis is also shown (dotted). Fig. 2, section through a closed lenticel from Winesap apple: hypodermal cells exposed are cutinized; cells farther below (dotted) contain tannin. Fig. 3, section through lenticel from Delicious apple: cuticle is uninterrupted across the lenticel. Fig. 4, surface view of epidermis over lenticel from Jonathan apple: guard cells have been broken apart. Fig. 5, section through closed lenticel from Black Twig apple: cuticle has filled the stoma. Fig. 6, section through lenticel from Rome Beauty: suberization of hypodermal cells is taking place but not complete; such a lenticel is still functionally open although it probably would have closed soon. Fig. 7, section through closed lenticel from Rome Beauty apple: phellogen has produced a suberized layer of cells. Fig. 8, section through lenticel from Golden Delicious apple: cutinization has started over exposed hypodermal layer. Fig. 9, section through lenticel from Jonathan apple: a crack has developed which extends from the outside to the large storage cells of parenchyma.

movement of substances. As the apple matures further, more and more of these lenticels become closed functionally although the stomata remain open, until, in such late varieties as the Winesap, nearly all of the lenticels are closed. In fact it is not uncommon to find pomes with only one or two open lenticels.

The closing of lenticels is never associated with mere stomatal closure. The thousand or more lenticels examined never showed the guard cells to be closed; indeed they seemed to be open their maximum amount. Closing of the lenticel is brought about in one of several ways: (1) The cuticle may seal over the stomatal aperture and thus seal the lenticel (figs. 3, 5). (2) The stomatal passage may remain freely open but a cuticle develop over the substomatal layer of cells, thus closing the lenticels to free movements of gases (fig. 2). (3) The stomatal passage may remain freely open and no cuticle develop internally but the substomatal cells become suberized so effectively that even intercellular movement of water or gases is prevented (fig. 6). (4) The stomatal passage may remain freely open, or the epidermal layer with several hypodermal layers may be completely torn away, but the exposed cells develop a phellogen that results in a suberized layer that is quite impermeable both to water and free gas movement; this type is least common (fig. 7).

After the apples were treated in the dye solution, closed lenticels of groups (1) and (2) remained entirely uncolored. Those of groups (3) and (4) showed a surface absorption of the dye with no penetration into the parenchymatous tissue beneath. To test whether or not the lenticels were impermeable to water and gases under pressure, a portion of the skin of the apple (the skin includes the epidermis as well as several layers of hypodermal cells) was removed and placed between two perforated metal discs in such a way that the lenticel was set in the center of the perforation. These discs were then placed in a metal jacket so that air or water pressure could be used. Water was placed over the exposed lenticel and the pressure pump was started. The air was forced against the lenticel with a pressure of 25 pounds. In no case where closed lenticels were used did air bubble through the water even when the pressure was sustained for 30 minutes. Water was then placed inside the container against the lenticel and again the pressure of 25 pounds was developed, but

even after half an hour of such treatment none of the water was forced through. In other words, the closed lenticel appears to be of no significance in the exchange of gas or water. It is probable, of course, that $CO_2$ can diffuse through the closed lenticel as well as it can through the unbroken cuticle, but this at best is a slow process as will be shown later.

The open lenticels may likewise be of several types: (1) Those in which the stoma remains open with the substomatal tissue incompletely modified or with this tissue completely modified but with large intercellular spaces (fig. 8); this type is found commonly in the very young pomes; (2) Those in which the lenticel had been firmly closed but which have been broken by the tension developed as the apple increased in size. Many times this rupture or tear extends to the very large parenchymatous cells of the storage tissue (fig. 9); (3) Those which are in various stages of repair following such a rupture (fig. 8). It appears that such breaks are repaired rather rapidly in the young apple, but as maturity approaches such rents are less and less easily covered.

### Number of lenticels per apple

The number of lenticels found on each apple seems in very broad terms to be a characteristic of the variety, with certain variations caused by the ecological conditions under which the apple is produced. The results of actual counts of the lenticels for the varieties studied are given in table I. Eight to ten apples of each variety were used in these counts.

Table I shows the variations one may expect to find among the varieties. To be sure the variation within a variety is likewise great. The intravarietal variation is proportionally the same in those varieties having a small number of lenticels, such as Winesap, as compared with those having a large number of lenticels, such as Spitzenburger. In Winesap the lenticel counts may average 536, but one apple in ten will show a total count of 450 while another will have as many as 800. Of these, all may be closed or many may be open. These variations are probably explainable on the basis of the ecology of the particular place on a particular tree. As will be seen later, when great care is used in selecting apples from trees, varia-

tion in the number of total lenticels as well as in the distribution of these between open and closed lenticel counts is materially reduced. In such varieties as the Spitzenburger, where the lenticel count is large, the range may be from 1500 to 2500 with correspondingly large variations in the number of closed and open lenticels. Even with this large variation, however, it appears that the lenticel number is something of a varietal characteristic. Inasmuch as the lenticel number seems somewhat related to the size of the apple, one

TABLE I

NUMBER OF LENTICELS PER APPLE

| HORTICULTURAL VARIETY | NUMBER OF OPEN LENTICELS (AVERAGE) | NUMBER OF CLOSED LENTICELS (AVERAGE) | TOTAL NUMBER OF LENTICELS (AVERAGE) |
|---|---|---|---|
| Winesap | 121 | 414 | 536 |
| Rome Beauty | 165 | 388 | 553 |
| Delicious | 66 | 737 | 803 |
| Golden Delicious | 187 | 656 | 843 |
| McIntosh | 200 | 651 | 851 |
| Black Stayman | 46 | 906 | 952 |
| Pearmain | 121 | 930 | 1051 |
| Black Twig | 185 | 903 | 1088 |
| Yellow Newtown | 78 | 1106 | 1184 |
| Starking Delicious | 92 | 1221 | 1313 |
| Red Delicious | 78 | 1274 | 1379 |
| Winter Banana | 170 | 1909 | 2079 |
| Spitzenburger | 211 | 1985 | 2196 |

should expect some correlation between it and the variety. In table II are given a few data indicating the relationship between the size of the apple and the number of lenticels within a variety. Thus the smallest five apples average 499 lenticels while the heaviest five average 572 lenticels. Although there seems to be a rather definite relationship, there are exceptions to the generalization. It is probable that this variation is determined by the time the development of the apple has reached its fourth week. Weights of the apple are reported rather than areas, since a close correlation exists between the two and weights are determined with greater accuracy and facility than are areas.

Although there is considerable variation among varieties so far as

the total number of lenticels is concerned, the distribution of lenticels over the surface of the apple is fairly uniform. The varieties studied showed approximately one-third of their lenticels over the stem two-thirds of the apple, with the remaining two-thirds of the lenticels crowded into the calyx one-third of the apple. Generally most of the open lenticels are to be found among the lenticels on the enlarged portion of the mature apple.

To summarize, the morphology of lenticels is much the same in the varieties studied. Actually these structures are not similar to

TABLE II

RELATION OF SIZE OF WINESAP APPLE TO NUMBER OF LENTICELS

| APPLE NO. | WEIGHT (GM.) | NUMBER OF OPEN LENTICELS | NUMBER OF CLOSED LENTICELS | TOTAL NUMBER OF LENTICELS |
|---|---|---|---|---|
| 1 | 152.5 | 59 | 435 | 494 |
| 2 | 157.2 | 170 | 287 | 457 |
| 3 | 160.5 | 99 | 422 | 523 |
| 4 | 168.5 | 138 | 406 | 544 |
| 5 | 170.0 | 76 | 399 | 475 |
| 6 | 184.5 | 128 | 470 | 598 |
| 7 | 185.7 | 129 | 478 | 607 |
| 8 | 211.5 | 102 | 410 | 512 |
| 9 | 232.9 | 133 | 312 | 545 |
| 10 | 260.0 | 178 | 422 | 600 |

the lenticels of woody stems. It is only an occasional pome lenticel which shows the development of a distinct phellogen. The large majority of apple lenticels are merely spots underneath a stomatal opening or an opening left by a fallen trichome or break, owing to the modification of the subepidermal cells. These lenticels may be open to mass movements of liquid or gases, or they may be closed in various ways so that neither liquids nor gases can be forced through as such, even under pressure. The number of lenticels per apple varies widely within the variety although within wide limits each variety has its characteristic range. The number of open lenticels per apple ranges from a few in such late varieties as Winesap to several hundred in others.

### Factors influencing number and type of lenticel

Although the number of lenticels varies within limits characteristic of the variety, various ecological factors seem to modify these ranges. It appears, furthermore, that the influences which are effective must act before the pome has become more than two to four weeks old. At this time it seems that the stomata as well as the trichomes are no longer formed, and, as already shown, lenticels are developments of these structures.

Unfortunately apples from variously fertilized plots were not available, but it was possible to obtain Winesap and Delicious apples from irrigation plots at Prosser, Washington. One group of

TABLE III

Lenticel counts of Winesap apples grown under two conditions of irrigation

| Treatment | Weight of apples (gm.) | Number of open lenticels | Number of closed lenticels | Total number of lenticels |
|---|---|---|---|---|
| 30 inches of irrigation..... | 188.23 | 121.2 | 414.1 | 535.4 |
| 60 inches of irrigation..... | 202.14 | 247.4 | 404.9 | 652.3 |

trees was irrigated at the rate of 30 inches of water per season and another was irrigated at the rate of 60 inches. Lenticel counts were made on ten apples of the Winesap variety per plot. The results are recorded in table III. These results indicate that Winesap apples grown under greater moisture conditions have a greater number of lenticels than when grown under 30 inches of irrigation. The difference cannot be accounted for on the basis of area alone. Further, the difference in the number of lenticels in these two plots is in the number of open lenticels.

Similar plots of Delicious apples were being conducted under the two conditions of irrigation. The lenticel counts of these apples are recorded in table IV.

The data in table IV indicate a response of Delicious apples which is opposite to that shown by the Winesap apples. Thus even though the apples receiving more water were larger than the others, the total number of lenticels on these averaged 69.4 lenticels less than

those on apples grown with 30 inches of water. If the results reported in these tables are significant, then the response of apples with respect to lenticel numbers and irrigation is varietal rather than general.

### Factors influencing closing of lenticels

Delicious and Winesap apples were used in this study. Pickings of apples were made at two periods: (1) two weeks before the usual harvest period,—these were called "early picking," and (2) at the usual harvest period,—these were called "prime picking." As soon

TABLE IV

LENTICEL COUNTS OF DELICIOUS APPLES GROWN UNDER TWO CONDITIONS OF IRRIGATION

| Tree | Treatment | No. of apples used | Weight (gm.) | No. of open lenticels | No. of closed lenticels | Total no. of lenticels |
|---|---|---|---|---|---|---|
| B-8..... | 30 inches | 15 | 255.4 | 141.2 | 1343.2 | 1484.4 |
| C-8..... | 30 inches | 15 | 235.5 | 169.3 | 1351.3 | 1520.6 |
| D-8..... | 30 inches | 18 | 236.2 | 110.2 | 1192.4 | 1302.6 |
| Average of 48 apples | | ..... | 242.4 | 142.2 | 1295.6 | 1435.8 |
| B-12..... | 60 inches | 15 | 282.9 | 132.0 | 1244.1 | 1376.1 |
| C-12..... | 60 inches | 15 | 283.2 | 111.8 | 1237.8 | 1349.6 |
| D-12..... | 60 inches | 15 | 279.9 | 116.6 | 1256.9 | 1373.5 |
| Average of 45 apples | | ..... | 282.0 | 120.1 | 1264.3 | 1366.4 |

as the apples were brought into the laboratory, some were subjected to the dye treatment and lenticel counts were made. Some were placed in ventilated containers at a temperature of 30° C. and relative humidity of 5–8 per cent. These apples were kept in this container for a week. Other lots were placed in other containers the temperature of which fluctuated between 10° and 15° C. and the humidity between 60 and 70 per cent. Still other lots were placed in desiccators over $CaCl_2$ at a temperature of 25° C. After the experimental period, the apples were subjected to the dye treatment and lenticel counts were made. The results are given in table V.

Winesap apples of both early and prime pickings were studied in a manner similar to the preceding series. The results are recorded in table VI.

It is apparent from these two tables that lenticels can be closed by any treatment which favors dehydration. Thus when apples are placed in a desiccator over $CaCl_2$ and at moderate temperature, nearly all of the lenticels are closed. Prime picked apples are less responsive than those picked earlier. It is to be noted that this closing effect obtains while the apples are still on the tree between the time of the early and prime pickings. The apples of the prime picking have about one-fourth the open lenticels of the previous picking.

TABLE V

EFFECT OF TEMPERATURE AND HUMIDITY ON LENTICEL OF DELICIOUS APPLES

| TREATMENT | NUMBER OF APPLES | NUMBER OF OPEN LENTICELS PER APPLE |
|---|---|---|
| EARLY PICKING | | |
| Untreated apples | 28 | 84.4 |
| Apples kept at 30° C. and 8–10% humidity for 1 week | 47 | 4.9 |
| Apples kept in desiccator at 25° C. for 1 week | 12 | 1.8 |
| Apples kept at 10°–15° C. and 60–70% humidity for 1 week | 65 | 52.9 |
| PRIME PICKING | | |
| Untreated apples | 31 | 49.3 |
| Apples kept at 30° C. and 8–10% humidity for 1 week | 24 | 23.04 |

This effect does not continue after the prime picking date, for if the apples are left on the tree they develop a large number of cracked lenticels or parts of the epidermis. This may be a response to water relations, for after some of the fruit is picked or has fallen, those apples remaining may absorb quantities of water sufficient to cause enlargement of the fruit, resulting in the cracking of the epidermis.

Another series was studied to determine whether apples which had been in storage until December 15 were still capable of closing their lenticels. Delicious apples were used. One lot was treated with dye solution as soon as it was taken from storage, and 51 apples of this lot averaged 48.04 open lenticels. A second lot was placed in a container kept at 30° C. and 8–10 per cent humidity, and 25 apples in this lot averaged 51.4 open lenticels. A third lot was treated as

lot 2 for one week and then was transferred to another container with a temperature of 15° C. and 60–70 per cent humidity for six weeks and 43 apples so treated averaged 49.9 open lenticels. A fourth lot was taken from storage and kept at 15° C. and 60–70 per cent humidity for six weeks, and 48 apples so treated averaged 33.7 open lenticels.

Thus it appears that, in storage, the senescence of apples increases to the point where a marked decrease in the reactivity of lenticels is

TABLE VI

EFFECT OF TEMPERATURE AND HUMIDITY ON LENTICELS OF WINESAP APPLES

| TREATMENT | NUMBER OF APPLES | NUMBER OF OPEN LENTICELS PER APPLE |
|---|---|---|
| EARLY PICKING | | |
| No treatment | 62 | 49.5 |
| Apples held 2 weeks at 30° C. and 5–8% humidity | 65 | 5.4 |
| Apples held 2 weeks at 25° C. in desiccator over $CaCl_2$ | 12 | 0.66 |
| Apples held 2 weeks at 15° C. and 60–70% humidity | 69 | 33.72 |
| PRIME PICKING | | |
| No treatment | 47 | 13.6 |
| Apples held 2 weeks at 30° C. and 8–10% humidity | 95 | 3.5 |
| Apples held 2 weeks at 15° C. and 60–70% humidity | 73 | 10.1 |

to be noted. It is only in lot 4 that any significant change has taken place. Further, no change takes place during the storage period of these apples. The apples placed in storage were of the same lot as those reported in table V of the prime picking series. When these apples went into storage they averaged 49.3 open lenticels and when they came out they averaged 48.04.

To summarize, it appears that the less mature apples respond more rapidly to treatments which induce lenticel closure than do mature apples. After a period in storage, Delicious apples fail to respond to treatment except after prolonged periods. The same type of closing takes place on the tree up to maturity, after which it appears that the lenticels are broken open by the enlarging fruit.

### RATE AND COURSE OF GASEOUS DIFFUSION FROM APPLES

Delicious apples taken from storage were used in these studies. They were taken from 1° C. and transferred as rapidly as possible to a chamber in a gas chain held at 25° C. Carbon dioxide-free air was drawn through the chamber and the carbon dioxide released by the apple was collected in absorption bulbs charged with KOH solution after the gas was dried by sulphuric acid. The air was moved through rapidly enough to prevent a "piling up" of the gas in the chamber. Weighings were made once each hour. This was continued until the hourly losses showed no appreciable variations.

It was necessary to determine the time required for the temperature of the apple to reach equilibrium with the warm outside temperature. This figure was obtained by thrusting a thermometer bulb into the flesh of the apple and making readings until the temperature of the apple was within half a degree of the air temperature. A typical series follows: at 4:00 P.M. the temperature of the apple was 1.0° C.; at 4:20, 3.0° C.; at 4:31, 4.0° C.; at 4:37, 5.0° C.; at 4:57, 9.0° C.; at 5:19, 12.5° C.; at 6:30, 20.0° C.; at 6:53, 22.5° C.; and at 7:50, 24.0° C. The air temperature was 24.5° C. Thus in 3 hours and 30 minutes the internal temperature of the apple was within a half degree of the temperature of the room.

Five Delicious apples were used in this test. It was hoped that after the gas determinations were complete and the number of open lenticels determined that sufficient variation in the number of open lenticels would exist to throw some light on the importance of these structures in gaseous diffusion. Fortunately one apple had only two open lenticels and another had 87. The first apple weighed 319 gm., the second 333 gm. $CO_2$ loss from these apples was practically identical. The amount of the gas lost during the test was slightly more than was calculated to give a saturated solution of it in the apple at the beginning of the experiment. The time necessary for the gas to come to equilibrium with the new temperature was 52 hours in the case of the apple with two open lenticels, and 49 hours in the case of the apple with 87 open lenticels. The $CO_2$ release in all the apples studied began very slowly (0.0008 gm. per hour) and rose more or less uniformly until a maximum of 0.0221 gm. per hour was reached at the end of 24 hours. The drop to equilibrium,

in about 24 hours more, was nearly a mirror image of the rise. It is obvious from these results that open lenticels play a very small part in the exchange of gases. It appears likely that the gas moves through the cuticle directly. If this is true, one might expect a slow movement through the closed lenticels as well, but surely these structures cannot be regarded as special organs for this function.

The importance of the cavity leading from the stem tip to the calyx end of the apple in gaseous exchange has been variously regarded. HAYLETT reports that it is insignificant in this regard. However, apples were studied by taking them from storage and plunging them into water heated to 60° C. Bubbles of the gas formed over the surface of the apple with no preference shown to lenticels, unless these were obviously open. There was vigorous bubbling from the calyx end of the apple. Whether this is a fair test or not is a matter for conjecture, but it is probable that when the gas pressure within the apple is large (as it would be if the apple is changed from 1° to 60° C.), the course would be as just noted. Further work will be done to determine the course during normal pressures.

When Winesap apples which averaged only 96 gm. per apple were used instead of Delicious, equilibrium time averaged 51 hours. Thus although these apples were approximately one-third the size of the Delicious, the time for equalization of gas pressures was approximately the same. It must be understood, however, that resistance to the escape of gases offered by the two varieties is probably quite different.

It is to be noted, finally, that moisture is lost from open lenticels from their immediate environs. When apples are allowed to stand in dry air for a few days, some lenticels are to be found in small pitlike depressions. These lenticels are open and the pit is formed as a result of water loss from the parenchymatous cells beneath. It is also probable that carbon dioxide produced in the vicinity of an open lenticel is lost through it.

## Summary

1. The development of the lenticels of 18 horticultural varieties of apples is traced. Lenticels may be open or closed depending on the character of the hypodermal cells. These may be cutinized or

suberized and thus rendered closed to the free movement of gases or liquids. Lenticels may also be closed when the stoma associated with the lenticel is closed over by means of the epidermal cuticle. Lenticels may be open when the hypodermal cells are unmodified or when the modified cells have been torn apart.

2. The total number of lenticels per apple is within certain wide ranges characteristic of the variety. The number may range from 450 to 800 in the case of Winesap apples and from 1500 to 2500 in the case of Spitzenburger apples.

3. The number of lenticels per apple may be varied by varying the amount of water available to the plant during the early development of the apples. This reaction is varietal rather than general. The Winesap apple when given more water produced more lenticels per apple than when grown with less water. The Delicious apples when given more water actually produced fewer lenticels even though the apples were larger.

4. Lenticels may be closed by processes which favor dehydration of the outer tissues of the apple. While the apples are still immature, they respond more completely to such treatment than do mature apples. After apples have been in storage for 6–8 weeks, they respond only after prolonged treatments.

5. Carbon dioxide gas within the apple escapes with equal speed whether the apple has many or few open lenticels. The period necessary for the gas to come to equilibrium with new temperatures is 49 to 52 hours.

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# MORPHOGENETIC STUDIES ON THE INFLORESCENCE OF COTTON

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 462

ULYS ROY GORE

(WITH FIFTY-THREE FIGURES)

## Introduction

A number of papers on the fruiting behavior of cotton have appeared (**3**, **1**, **17**). Work has been done also on the influence of ecological factors on fruiting behavior (**13**), the arrangement of parts in the cotton plant (**4**), regularity of blooming (**16**), lint development (**7**), microsporogenesis and chromosome numbers (**2**), megasporogenesis (**9**), and root stem transition (**21**). Many genetic studies dealing with inheritance of floral characters, fruiting behavior, and vegetative characters have been summarized by KEARNEY (**12**). The purpose of the present paper is to describe the ontogeny and development of the primary axis (main stem) and of the floral axis (fruiting branch), together with the developmental morphology of the flower and its vascular anatomy. An attempt is made also to interpret the androecial column on the basis of ontogenetic studies.

## Materials and methods

Material from cotton plants growing near Fayetteville, Arkansas, of the Pima, Sea Island, and Mebane varieties, was collected at various intervals throughout the summer of 1933. This was fixed and preserved in a modification of Navashin's solution consisting of equal parts of A (7 cc. glacial acetic acid, 1 gm. chromic acid crystals, 92 cc. distilled water) and B (30 cc. formalin and 70 cc. distilled water), mixed at the time of using and left in this solution until needed for study. For the anatomical details the material was imbedded following the n-butyl alcohol technique, sectioned at 15 $\mu$, and stained in safranin-gentian violet-orange combination. Both longisections and transections were made of the different bud clusters, but transections proved to be more valuable for interpreting the

method of development of the axes as well as for the vascular details of the flower.

It was found most convenient for a study of developing axes and floral ontogeny to make observations under a wide-field binocular microscope. For this study the subtending leaves with stipules were dissected away from the stem tip, exposing the young primordia; then, following the method used by LUYTEN (**15**), the primordia were stained with strong IKI, which facilitated the observations. Plants grown in the greenhouse in the spring of 1933 failed to produce more than a single flower to a fruiting branch and hence were not used in these studies.

## Investigation

### PRIMARY AXIS

DESCRIPTION.—The main stem consists characteristically of a single indeterminate primary axis from which the branches arise. With the exception of the cotyledons, the leaves occur on the main stem in a regular 3/8 spiral arrangement. Examples of variation from this arrangement are found in hybrid stocks, which may have a 1/3, 2/5, or 5/13 spiral arrangement. The Old World species have a 1/3 spiral leaf arrangement (**4**).

Two clearly distinct types of branches, differing in form and function, arise in the axil of the leaves on the main stem (**3, 4**). These are (1) vegetative branches similar in all respects, morphologically and functionally, to the primary axis; (2) fruiting branches which are different in form, function, and method of development.

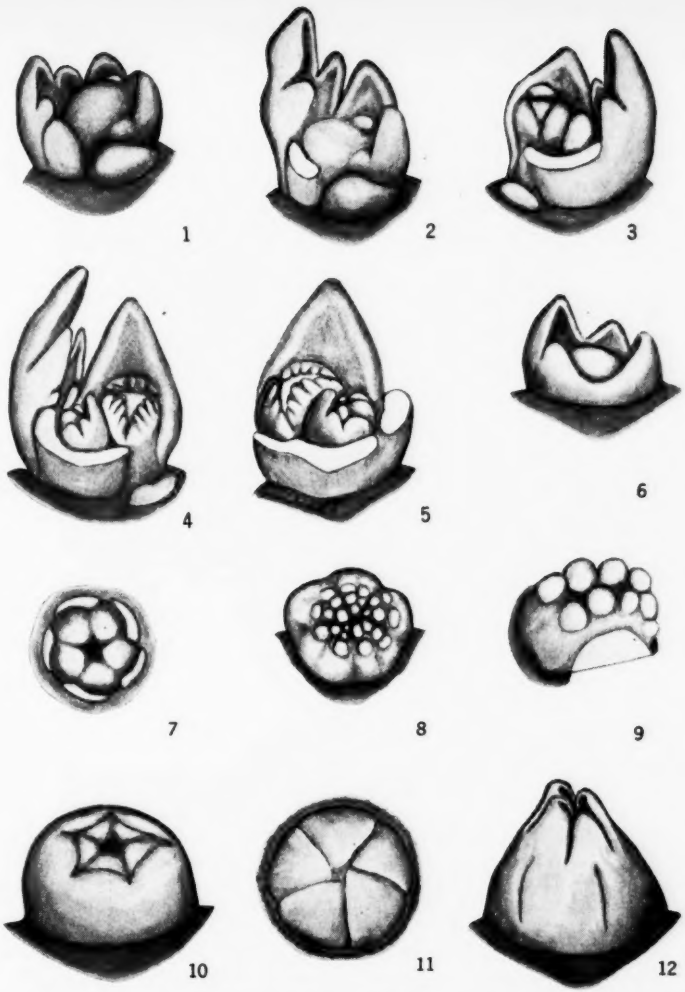
At a node on the main axis in Mebane, the following structures are generally found: a leaf with stipules, an axillary branch either fruiting or vegetative, and an accessory dormant bud. In Pima and Sea Island this accessory bud usually develops, but is soon abscissed. This accessory bud, when located at the base of a vegetative branch, is termed "extra-axillary" by COOK (**3**) and KING (**13**); and when located at the base of a fruiting branch, "axillary." According to GRAY (**10**) and GOEBEL (**8**), buds are normally either terminal or axillary, are usually single, and appear in the axil of the leaf. When more than one bud appears in the leaf axil (**10**) it is due to the fact

that the true axillary bud or primordium has by branching given rise to one or more extra buds.

ONTOGENETIC STUDIES.—The main stem and the vegetative branches are developed by continued activity of the terminal bud of each respective axis. Leaves with stipules and axillary bud primordia are developed in acropetal succession at each node from a terminal meristem. This method of growth is monopodial in character as contrasted with sympodial growth. The terminal growing point is surrounded and protected by the primordia of several leaves and stipules, these structures being covered with hairs which afford complete protection to the developing primordia.

The early development of the leaf and stipules proceeds as follows: the leaf primordium and stipule primordia arise practically simultaneously from the apical meristem of the primary axis (figs. 1, 2). The leaf primordium is rounded and collar-like at first, later tapering somewhat up to the rounded tip. As the embryonic leaf grows upward it becomes bluntly pointed and assumes the shape shown in figure 2. There is little evidence of differentiation into petiole and blade at this stage, except the slight beginning of the plicate folding characteristic of the lamina of a young leaf. No epidermal hairs are present at this stage, although they begin their development early. The third young leaf from the tip is completely covered with them.

The tip of this first protuberance becomes the median lobe of the palmate leaf; its base develops into the petiolar region. There soon develops on the slightly incurved margins of this primordium two pointed protuberances located about halfway between the tip and its base (fig. 1). These are the primordia of the two lateral lobes of the leaf. The young leaf, consisting of three lobes, now proceeds to develop in length; continued inward plicate folding, as the lobes increase laterally in size, forms a cuplike portion (figs. 2, 3). If additional lobes occur they arise in the same way and are similar in all respects (figs. 4, 5). Seven lobes is the usual number for leaves on the main axis, while on the fruiting branches of Pima and Sea Island varieties the leaves usually have three. In Mebane five-lobed leaves are commonly found on the fruiting branches. Soon the tips of the individual lobes meet and the lamina develops rapidly. The main veins appear as definite ridges on the abaxial surface of the leaf and




FIGS. 1–12.—Fig. 1, growing point of cotton showing early stages in ontogeny of leaf and stipules, axillary bud, lobes of young leaf, and general topography of tip of main axis, drawn from above and slightly to one side; Pima. Fig. 2, later stage in leaf ontogeny, one stipule cut away; Mebane. Fig. 3, general topography of fruiting branch terminal, origin of bracts on flower primordium and new axillary primordium; Sea Island. Fig. 4, later stage in ontogeny of sympodial axis showing slightly older floral bracts; Pima. Fig. 5, later stage than fig. 4 showing origin of new fruiting branch terminal between leaf and flower primordium; Mebane. Fig. 6, axillary bud of fruiting branch showing bractlike structure, growing point, leaf and stipule primordia of second node; Mebane. Fig. 7, early ontogenetic stage of staminal column; petal primordia well differentiated and showing alternate staminal column lobes; Sea Island. Fig. 8, later stage in ontogeny of staminal column showing stamen primordia in two rows on each ridge; Pima. Fig. 9, one of the five lobes of developing staminal column with stamen primordia, under greater magnification than fig. 8; Pima. Fig. 10, young calyx with bracts removed; Mebane. Fig. 11, developing petals; Pima. Fig. 12, young pistil; Mebane.

are very thick in proportion to the rest of the leaf. The venation is palmate.

Simultaneously with or shortly after the initial leaf primordium becomes distinct, two stipular flanking protuberances arise (fig. 2). These are pointed and concave at first, but as they grow they widen out from the base somewhat, later become toothed, fold inward very slightly, and keep pace with the growth of the leaf. They are not folded or plicate as is the young leaf but remain more or less flattened, their interlocking hairs holding them tightly appressed to the leaves.

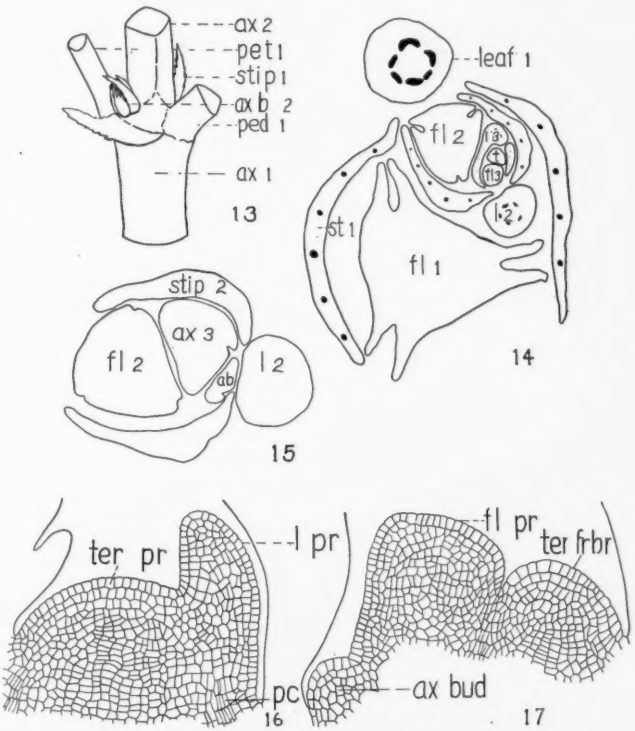
### Fruiting axis or sympodium

Description.—Casual examination of a fruiting branch shows that the flowers are not in the axil of a leaf (fig. 13), but appear to stand opposite the adjacent leaf. The internodes of this branch are slightly zigzag and the leaves appear as if alternately arranged. Detailed examination shows that this arrangement is the result of sympodial development. A sympodium, according to Gray (**10**) and Goebel (**8**), consists of a series of seemingly superposed internodes. Actually each internode (or several internodes) belongs to succeeding generations of axes. Such a sympodium is developed more or less as follows: From the axil of a leaf on a given axis there arises a new axis which by its growth pushes the terminal portion of the axis from which it arose to one side so that this once terminal portion may come to appear as if lateral in origin. Many examples of sympodial types of development are to be found (**8, 10**) in the Caryophyllaceae, Malvaceae, Solanaceae, Linaceae, etc.

The fruiting branches of all known cottons are now recognized as sympodial (**22**). This type of inflorescence was earlier termed a false raceme by Goebel (**8**), and was so applied to cotton by Balls (**1**). Leake (**14**) was among the first to group the Indian cottons on the basis of their secondary branches into monopodial and sympodial forms. He also observed that monopodial branches are usually ascending while sympodial branches are spreading. Cook (**3**) opposed the sympodial interpretation and termed the cotton inflorescence as either an "extra-axillary" branch or simply a fruiting branch. Cook and Meade (**4**) stated that the behavior of cluster cottons may indicate that the fruiting branches have a sympodial method of growth.

They speak also of the abortion of the terminal bud of the fruiting branch.

A single segment of a fruiting branch consists of an internode, a




FIGS. 13–17.—Fig. 13, sketch of portion of fruiting branch of Mebane showing parts of two sympodia; termination of axis 1 is the flower whose pedicel is shown, together with leaf and stipules: *ax 1*, *2*, axes 1 and 2; *pet 1*, petiole of axis 1; *ped 1*, pedicel of flower; *ax b 2*, axillary bud developed from axis 2. Fig. 14, outline of transection of terminal bud of fruiting branch showing three sympodia in cross section and terminal for fourth axis; axillary fruiting branch buds not shown: *fl*, *1*, *2*, *3*, *L 1*, *2*, *3*, *st 1* and *t*, flower, leaf, stipule, and terminal for respective sympodia. Fig. 15, lower level on sympodia 1 and 2, also greater magnification; axillary bud of axis 3 is shown: *ax 3*, axis 3; *ab 3*, axillary buds axis 3. Fig. 16, longisection cell drawing of terminal of main axis; Mebane: *ter pr*, terminal primordium of axis; *l pr*, leaf primordium; *pc*, procambium. Fig. 17, longisection cell drawing of terminal fruiting axis: *fl pr*, flower primordium; *ter fr br*, terminal fruiting branch; *ax bud*, axillary fruiting branch bud.

leaf with stipules, the flower bud, and two axillary buds one of which remains dormant and becomes the axillary bud of the fruiting branch while the other continues the secondary axis. The fruiting branch of cotton is made up of a series of these segments which gives to the entire structure a jointed appearance.

The importance of sympodial development from the economic point of view is related to two well known facts. First, flowering and fruiting are wholly dependent upon the production of sympodia. Certainly the number of potential flower buds produced, among other factors, influences the yield of cotton. Secondly, the time at which production of sympodia begins, whether early or late, determines earliness or lateness of fruiting. The early fruiting and maturing varieties begin the production of sympodia early, some even at the first or second node, and no vegetative branches are produced in some varieties. Pima produces a number of monopodial divergences before any sympodial divergences appear.

ONTOGENETIC STUDIES.—The first evidence of the development of a fruiting branch is a primordium which occurs in the axil of a leaf on the main stem at the second or third node back from the apical meristem (fig. 2). This axial primordium is early differentiated into two separate growing points: one grows faster and becomes the fruiting branch primordium; the other grows slowly and develops the axillary bud on the main axis, which is homologous with the axillary bud of the fruiting branch. The fruiting branch primordium becomes raised at three points where the first leaf and stipules are to arise. At this stage a single primordium exists which resembles the apical primordium of the main stem. After the leaf and stipule primordia are definitely differentiated it begins to grow away from the subtending leaf and is directed away at an increasing angle. It becomes bluntly conical in shape and projects beyond the subtending stipules (fig. 4), continuing its development into a flower bud. At this same time a zone of tissue becomes active between the leaf and the primordium of a flower (fig. 5). It soon develops two unequal-sized growing points, the smaller of which gives rise to the axillary bud; the larger one continues the fruiting branch by developing a leaf and stipules for the next axis. By a repetition of this process the fruiting branch comes to consist of a series of axes, each axis made up of a single in-

ternode and each terminated by a flower. Cottons in which a sympodium consists of more than one internode are exceptional. All cultivated American species have a single internode to each sympodium or axis (**22**). Early in the development of the fruiting branch, when the first fruiting axis is being formed, it is pushed to one side so that the developing structures are projected to one side of the leaf on the main stem. Thus a fruiting branch is more nearly horizontal with the primary axis and grows out either to the right or left of the subtending leaf on the main stem.

The arrangement of the succeeding axes is shown in figures 14 and 15, which are from transections of the tips of young fruiting branches of Mebane. Here are shown the parts of three successive axes. The older proximal axes project beyond the younger distal ones so that the petioles of the leaves on axes 2 and 3 are shown in transection. In a longisection of a tip of a fruiting branch (fig. 17) may be seen the flattened floral primordium, the axillary bud primordium, and the new terminal growing point located between the leaf and flower primordium.

### Axillary bud of fruiting branch

The behavior of the bud occurring in the axil of a leaf on a fruiting sympodium has been investigated by King (**13**) for Pima and Upland cottons. No detailed study of the ontogeny or the morphology of these structures is available, but they have been described as developing short vegetative branches by Cook (**3**) and as single floral buds without any subtending leaves or stipules by King (**13**).

By dissecting out the successive axillary buds on a fruiting branch it is possible to get all stages in their ontogeny and development, from the earliest primordium to the flower bud, leaf, and stipules. Axillary buds from fruiting branches of Pima, Sea Island, and Mebane were studied. Since Mebane has the greatest variation in structure it will be described first. It has its origin from the axillary primordium of the fruiting sympodium as already described. The first divergence from the axillary bud primordium is a bractlike structure, unattended by stipules, possibly representing a reduced leaf and stipules. There next arise a leaf and stipules for the second node, followed by the turning aside of the growing point and the develop-

ment of a sympodium. The new terminal primordium develops a monopodial axis at the next node, however, and at a succeeding node another sympodium may be developed. This alternate production of a sympodial axis and a monopodial axis may continue until three or four flower primordia are produced. As pointed out by KING (13), these axillary bud structures on Upland varieties usually remain dormant, until late in the season when proper conditions arise they develop branches; but only one of the flower buds functions. Figures 18–24 show consecutive cross sections of this bud in Mebane.

In Pima and Sea Island these axillary fruiting branch buds arise exactly as in Mebane. Their further development, however, is different. They are with respect to their mode of development similar to sympodia of the fruiting branches of these respective varieties. Each axis has its complement of leaf, stipules, flower bud, axillary bud, and terminal. The vascular structure of this axillary fruiting branch bud is like that of the fruiting axis and these studies show that in Pima and Sea Island most of these branches consist entirely of sympodia (figs. 25–33).

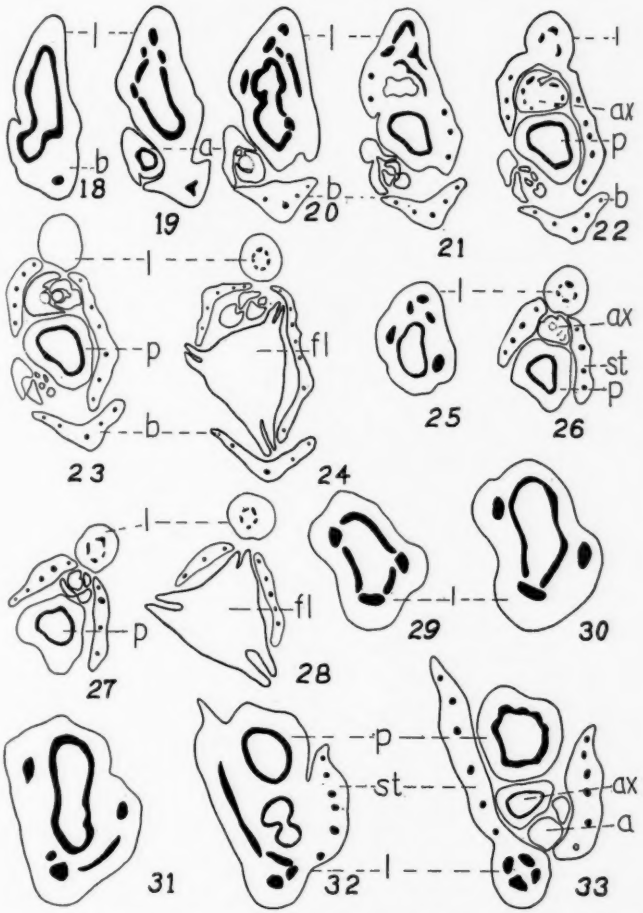
In Pima and Sea Island these axillary buds do not usually remain dormant, but develop shortly after they are formed, lengthening the first internode to 0.5 inch or longer. They usually do not grow long before they shrivel up and are abscissed. This confirms KING'S work on Pima. In Mebane these buds may develop a flower when the extra-axillary flower bud is shed, or late in the season they may produce short axillary branches. The causes for differences in behavior of these axillary fruiting branch buds of the different varieties are not known and need further investigation.

### COTTON FLOWER

DESCRIPTION.—The general features of the cotton flower and the arrangement of its various organs are described by BALLS (1), COOK and MEADE (4), and ROBBINS (18), so that only a brief summary is necessary here before taking up the ontogeny of the individual floral organs.

The flowers in the species studied are extra-axillary, terminal, and solitary, although they may appear as if arranged on alternating sides of the fruiting branch (fig. 13). There are five whorls or cycles

of organs: the first whorl consists of an epicalyx of three relatively large leaflike bracts; the next whorl or true calyx consists of five unequally lobed, mostly undiverged sepals; the third whorl, the corolla,




FIGS. 18–33.—Figs. 18–24, successive transections of axillary fruiting branch bud (Mebane) showing two types of axes; vascular details shown in black. Figs. 25–28, successive sections of same for Sea Island. Figs. 29–33, successive sections of same for Pima; note sympodial nature of bud.

consists of five obcordate petals, white in Mebane and yellow in Pima and Sea Island, with a purple spot on the claw of the petal in the latter variety. The petals are convolute in the bud and are held tightly folded together by epidermal hairs. The fourth whorl or androecium consists of a staminal column bearing ten more or less double rows of stamens with reniform two-loculed anthers. The last whorl or the pistil is made up of from three to five undiverged carpels. The fruit is a three to five loculed capsule or boll, dehiscent by splitting along the dorsal sutures of the carpels. The seeds are covered with lint hairs which are elongated epidermal cells of the outer integument (7).

ONTOGENETIC STUDIES.—The young flower becomes evident with the appearance of a bract primordium on the floral meristem. This first primordium is smooth margined, and appears opposite the leaf (fig. 3). The next two bracts follow in succession and are similar in shape to the first. The three bracts grow rapidly in size, and lobes, which become the teeth of the mature bracts, are evident early. The floral bud is now triangular in shape.

When the bracts reach the stage shown in figure 5, the primordium of the calyx arises. This primordium consists at first of an undulating collar or meristematic zone which, as it grows upward, grows more rapidly at five definite points. These points become the tips of the unequally five lobed gamosepalous calyx. The rounded lobes of the calyx become folded over and inward (fig. 10), and for a time cover almost completely the remaining developing floral organs. Both the bracts and calyx are persistent, remaining green until the boll ripens.

After the sepal primordia arise there remains a rounded mass of meristematic tissue at the center. With subsequent growth of this mass its outer margin becomes raised, leaving a depression. This now collar-like ring is the beginning of the common stamen-petal zone or primordium (fig. 10). The ring soon becomes noticeably elevated at five points on its rim, at an earlier stage than that shown in figure 10, and five lobes arise simultaneously on its lateral face. These thickened regions alternate with the sepal lobes and are the petal primordia.

The five petal primordia become flattened as they grow out from

the zonal meristem, increasing in size both laterally and vertically. An apical notch occurs when the petal is very young. The petals grow more at the top than at the base, as seen in figure 11, and so become convolute in the bud. Continuing their growth in height they soon push up and overtop the young staminal column. They

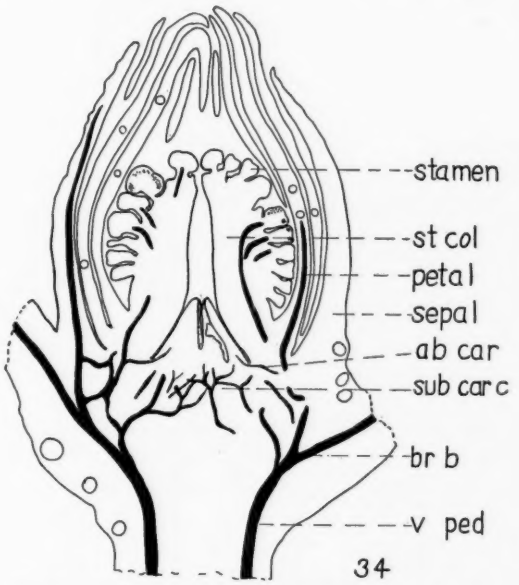


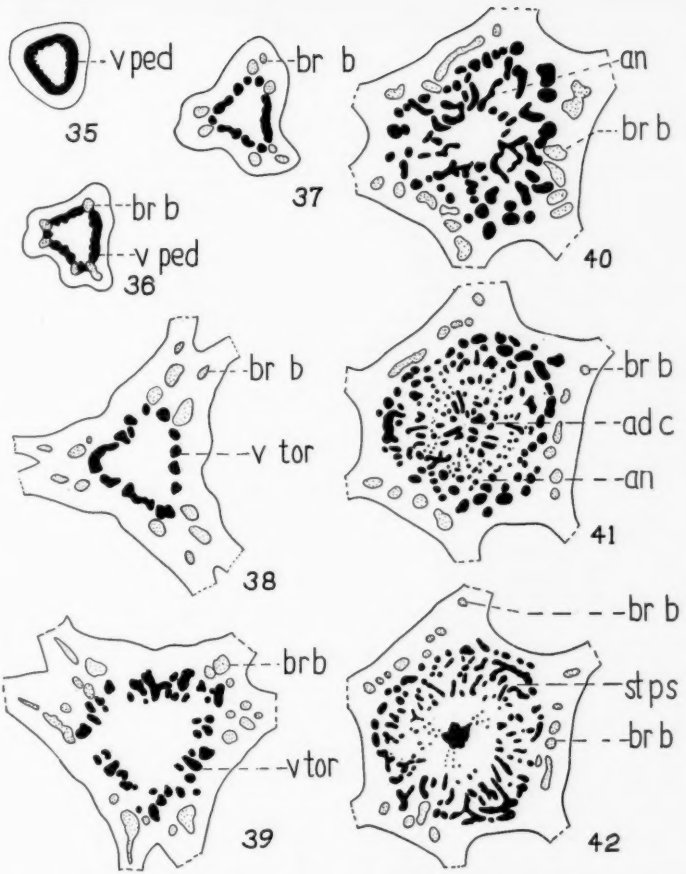

FIG. 34.—Nearly median longisection flower bud of Mebane showing vascular supply to various organs at this stage of development: *v ped*, vascular supply to pedicel; *br b*, bract bundle; *sub car c*, subcarpellary complex; *ab car*, abaxial carpellary bundle; *st col*, staminal column.

overlap, either to the right or left, and are held together in the bud by interlocking hairs. After becoming folded over one another, a further upward growth brings their tips out beyond the sepals as a conical-shaped structure, with the sepal tips of the calyx cup tightly appressed around the corolla (fig. 34).

It was just stated that the petals and stamens develop from a common zone of embryonic tissue. The first evidence of divergence of the staminal column occurs before or about the time petal primordia

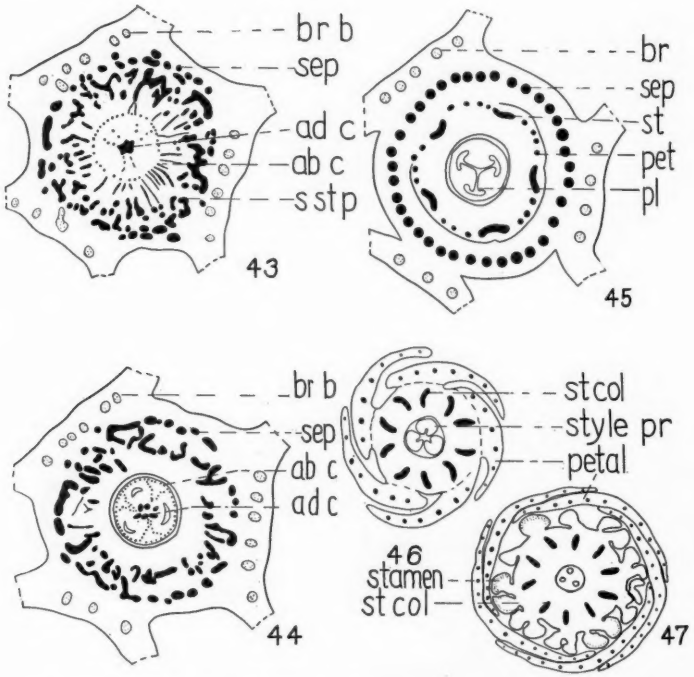
are diverged, when five distinctly higher points appear on the upper and inner margin of this ring, and occupy most of the rim, with slight depressions between each of them (fig. 7). The inner edges now appear most active and grow inward for a time. On each of these five primordial lobes there begin to appear rounded primordia of stamens directed toward the center on the inner face of each lobe. Figure 8 is of a developing staminal column of a slightly later stage, for here ten rows or ridges of stamen primordia can be seen, with deeper furrows between pairs of them. The original five lobes shown in figure 7 can still be recognized, however. It is significant that the staminal column lobes alternate with the petals at this stage, but with later growth and twisting of the petals the staminal column lobes appear opposite the petals. Soon after the first spherical stamen primordia arise, other primordia appear on top and down the outside face of each lobe of the staminal column (fig. 9), until ten definite rows are formed. Each of these ten rows of stamen primordia, except the bottom members of each row, divides one or more times. Multiplication of stamens by division of existing primordia produces a large number of stamens, from 50 to 125 or more in the varieties studied. Branching of stamen primordia occurs at the tip of the staminal column as well as along the sides. This multiplication of the members of a whorl of floral organs is termed "deduplication" or "chorisis" (**8, 10**), and occurs in the Tiliaceae and Hypericaceae (**5**) as well as in the Malvaceae. The young anthers are at first spherical, but later become two lobed and reniform in shape. The short filaments bearing the capitate anthers grow little more until a few days before flowering, when they increase greatly in length. The fully formed stamens in a flower bud have the appearance of a rounded compact mass of anthers. The top of the staminal tube remains definitely five lobed at maturity.

In the cavity in the center of the axis which has been overtopped by the staminal column, the carpel primordia arise. They are first evident as a zonal meristem slightly raised at three to five definite points. These points appear very early and become the stylar projections. Their further growth is extremely slow in the early stages as compared with the growth of the rest of the young pistil (fig. 12). The upward growth of the rim of the ovary brings the styles to project into the staminal tube at an early age.




FIGS. 35–42.—Successive transections of flower bud showing vascular supply and various divergences at successively higher levels; Pima. Fig. 35, base of pedicel. Fig. 36, beginning of divergence of bract bundles; bract bundles stippled, rest of system solid black. Fig. 37, level of divergence of six bract bundles and breaking up of rest of cylinder. Fig. 38, further breaking up of bract bundles and widening out of remaining vascular bundles. Fig. 39, further outward divergence and anastomosing of bundles. Fig. 40, region of anastomosis or subcarpellary complex, with inward divergence of adaxial carpellary bundles and rest of system finely anastomosed. Fig. 41, adaxial carpel bundles at center of toral region; general anastomosis of bundles still evident. Fig. 42, adaxial group now a solid structure in the center: *v ped*, pedicel vascular supply; *br b*, bract bundles, also stippled; *v tor*, toral bundles; *an* region of anastomosis; *ad c*, adaxial carpellary system; *st p s*, stamen-petal-sepal region of anastomosis. Figs. 35–47 drawn to same scale.

Simultaneously with increase in size of the ovary, outgrowths are produced inside which, on growing inward and upward, develop into




FIGS. 43–47.—Continuation of figs. 35–42; Pima. Fig. 43, region of anastomosis with inward divergence of many abaxial carpel bundles. Fig. 44, adaxial carpel bundles undiverged and two going to each carpel; stamen-petal group diverging inward from the still anastomosed sepal-petal-stamen complex which has continued undiverged from carpel base. Fig. 45, sepal ring completely separated from stamen-petal ring. Fig. 46, higher level on stamen-petal column. Fig. 47, region of complete divergence of all floral organs: *sep*, sepal bundle; *pet*, petal bundle; *s st p*, sepal-stamen-petal bundles; *st col*, staminal column bundle; *ab c*, abaxial carpellary bundles; *style pr*, style primordia; *pl*, placental ridges of septum.

the true septa on which the placental ridges are found (fig. 44). These septa arise separately and remain distinct for some time, finally coming together and touching in the center; but their edges never fuse completely except near the top. From the two vertical

edges of each septum the rounded placental ridges appear. The edges of these placental masses grow outward at an increasing angle toward the external carpel wall, finally becoming a flattened mass of meristematic tissue. On the placenta next arise the rounded ovule primordia. The development of the ovule has been traced in a previous paper (9) and will not be repeated here. As the ovules develop and the ovary enlarges, the edges of the placenta become closely appressed to the edges of adjacent septa.

The growing point of the axis can be seen in median longisections of young ovaries located in the center of the converging septa. It is possible that the height to which the end of the axis extends in the boll has something to do with incomplete opening of the boll at maturity. Externally the ovary increases greatly in diameter, bulging somewhat as it grows in height, and thus appears as a conical structure, bearing at the tip of each carpel a style.

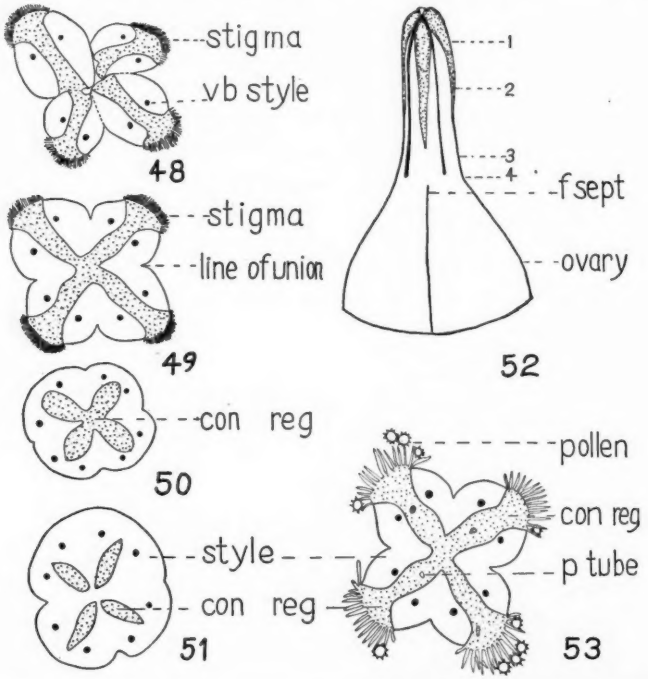
The rapid increase in girth of the ovary portion comes about through the early origin and functioning of the false septum of each carpel, which in this case functions as a cambium. This layer later becomes a line of weakness in the ripe capsule, thus accounting for the loculicidal dehiscence of the fruit. The stylar projections early become folded laterally and remain folded as they grow in length. Their edges meet those of adjacent styles and complete coalescence of the tissues occurs, so that the locular cavities are completely closed at the top.

The tips of the styles continue to grow in length and several days before flowering project into the staminal tube. A stigmatic and pollen tube conducting region is early differentiated and occupies a median position with respect to the style as a whole, fitting like a wedge into the style and exposing only a lateral stigmatic surface (figs. 48–51). Stigmatic hairs develop only on the exposed lateral surface of the pollen tube conducting region (fig. 52). The styles of Pima and Sea Island are proportionately long and project beyond the anthers for perhaps half their length. Those of Mebane extend out slightly, if any, beyond the anthers. It is recognized that the distance which the stigmas project beyond the stamens has a great deal to do with the amount of natural crossing occurring in cotton.

ANATOMY.—The nodes on the main axis and branch axes have

three traces, which agrees with the general condition in the Malvaceae as reported by SINNOTT (19).

The anatomy of the pedicel as in other plants (6) is essentially that of a young stem. The primary vascular cylinder consists of




FIGS. 48–53.—Figs. 48–51, successive transections of stigma and style of Mebane. Fig. 52, habit sketch of half-grown pistil; stigmatic region stippled: *1*, *2*, *3*, *4*, levels of figs. 48, 49, 50, and 51. Fig. 53, transection of upper part of styles of Upland cotton, taken day of blooming; note pollen tubes in conducting region: *con reg*, pollen tube conducting region; *p tube*, pollen tube; *f sept*, false septum.

twenty to thirty bundles arranged in a circle, each separated by rays. At the place where the enlargement of the torus begins, the pedicel becomes triangular and the vascular circle widens out at three definite points, also becoming triangular in outline (fig. 36). At a higher level two bundles diverge outward at each corner of this triangle and proceed to the outer and lowest edges of the bracts (fig. 37).

As the definitely enlarged toral part of the flower is reached other bundles diverge from the system and go to the bracts (fig. 39). At this level the entire vascular structure becomes somewhat funnel-shaped by the outward divergence of the whole system, a large pith region remaining.

At a higher level (fig. 40) several bundles from this system begin to diverge inward toward the center, while at the same time some of the bundles diverge toward the outside. These bundles, at first fairly distinct, soon anastomose to form a network of smaller bundles. The bundles comprising the inner portion of this region of anastomosis, or subcarpellary complex as it may be termed at this level, begin to diverge inward still more, as shown in figure 41. Most of the bract bundles have progressively diverged by the time this level is reached. Extensions of the inner diverging portion become the adaxial carpellary system or placental bundles. This anastomosing zone continues upward and diverges still farther inward. Figure 42 at a slightly higher level shows the large adaxial group, which is now one large bundle at the center of the torus, from which small bundles diverge and extend into the septa of the carpels. This main large adaxial bundle extends for only a short distance, however, before it is diverged into two distinct adaxial bundles to each carpel. From these adaxial bundles, as they continue upward in the boll, there extends a bundle to each of the ovules. The remnants of the adaxial system continue into the style.

The anastomosed sepal-petal-stamen bundles (fig. 42) continue upward for a short distance before the abaxial carpellary bundles are diverged (fig. 43). They extend inward almost horizontally and supply the outer carpel wall. There are several of these abaxial bundles supplying the outer wall of the boll, and their number varies with the number of carpels constituting the ovary. There are usually ten or more to each carpel. With the divergence of the abaxial bundles the carpellary system is complete. The false septum of each carpel consists of meristematic parenchyma and extends vertically in a median plane in each carpel. The anastomosed abaxial system of each carpel is thus separated by the false septum.

The central portion of each stylar segment consists of glandular tissue. Since the styles are fused together there appears a central re-

gion with three to five radiating areas (figs. 48–51). This region is continuous with the placenta of each carpel (5) and it is through this tissue that the pollen tubes pass to the micropyle of the ovule. This central pollen-tube conducting region is ensheathed by several layers of parenchyma in which the adaxial bundles are located. The unicellular stigmatic papillae are found on the surface wherever the pollen-tube conducting region is exposed (figs. 48–53).

Practically all the bundles supplying the bracts have diverged at the level of figure 44, although the bracts themselves are not distinct from the sepals until at a higher level. These bundles bifurcate until there are fifteen or more supplying each bract, the main veins extending to the tips of the teeth.

The anastomosed sepal-petal-stamen group of bundles continues undiverged for some distance above the separation from the carpels. At a higher level (fig. 45) the stamen-petal bundles diverge inward, leaving externally the ring of bundles which supplies the calyx cup. The sepal ring of bundles branch laterally somewhat, but chiefly continue on straight to the tips of the sepals.

The stamen-petal ring now consists of five large bundles that alternate with five groups of smaller ones, the petal bundles. The five large ones are the staminal column bundles. These five bundles, which can now definitely be called staminal column traces, extend farther inward and each divides into two equal sized bundles that come to lie opposed to each other, thus forming ten main radially arranged staminal column bundles which continue into the staminal column. The distance between two related bundles is increased by the development of a fleshy column, so that neither bundle now alternates with the petals but both appear opposite the petals. As these ten bundles ascend in the column, small bundles are given off from their outer edges to each stamen. These small stamen traces may branch one or more times, in keeping with branching of the stamens. One trace supplies an individual stamen. Ten principal bundles are constant for the staminal column of the three varieties studied. This number is also regularly found in *Malva* (20) and *Hibiscus* (11).

The petalline bundles, five groups of three or more each, soon branch and rebranch several times, and with considerable anasto-

mosing continue to the margins of the petals. The vein supply of the petals is similar in many respects to that of the calyx and bracts.

## Summary

1. The ontogeny and development of the main axis, the fruiting branch, and the flower of three varieties of cotton are described. The vascular anatomy of the flower is also given.

2. The main stem consists of a single indeterminate primary axis from which leaves, stipules, and branches arise in acropetal succession from a terminal meristem.

3. The fruiting branch develops sympodially, and consists of a series of single internodal axes. As each axis is terminated by a flower, a new bud arises to continue the branch. This process is repeated each time a flower is formed.

4. From the flower primordium there arise three bract primordia, a sepal zone, a common petal-stamen zone from which stamens and petals are diverged almost simultaneously, followed by the carpel primordia in the order named.

5. The anomalous staminal column is developed from a basic number of five stamens, each proliferating to form a considerable number of stamen primordia on each of the five lobes of the column. Branching of stamen primordia further increases the number of stamens.

6. Three traces supply the nodes on the main axis and branch axes.

7. The vascular cylinder of the flower becomes anastomosed in the toral region. From this there is successively diverged: first, six bundles to the bracts, then the adaxial carpellary system, followed by the abaxial carpellary system at a higher level; next, five or more bundles supply each sepal, three or more bundles to each petal, and finally ten main bundles to the staminal column.

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# HISTOLOGICAL VARIATIONS IN COSMOS IN RELATION TO PHOTOPERIODISM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 463

ORLIN BIDDULPH

(WITH TWENTY-NINE FIGURES)

## Introduction

GARNER and ALLARD (**8, 9, 10**) have described the response of *Cosmos* when subjected to various photoperiodic treatments. Their investigations show that *C. sulphureus* Cav. is a short day plant growing vegetatively in long days and flowering when the daily light period is shortened to ten hours. It can be grown to a height of 15 feet or more or made to flower in 50 days, depending on the length of the daily light period. The age and size of the plants make no difference in the speed with which they react to the change in the length of the daily light period. By the use of an electric light to prolong the light period of the normal day, it was found that an intensity of 4-foot candles was effective in producing vegetative growth in short day plants and reproductive activity in long day plants.

When short day plants which have been given a 10-hour day for 15 days are transferred to conditions of a long day, only a few of the blossom buds already initiated finally open, the others being permanently suppressed. Vegetative shoots from the axillary buds on the axis below the apical flower bud soon begin to elongate and the latter is apparently deprived of the necessary nutritive materials for development.

GARNER, ALLARD, and BACON (**11**) have applied the concept of the carbohydrate-nitrogen relation as a factor in flowering and reproduction (**16**) to photoperiodic response, and have found that a change in soluble carbohydrate accompanies a change in the length of the daily light period. In *Cosmos* there is an increase in reducing sugars in the apex of the stem within 48 hours after the transfer of the plant from long to short days.

NIGHTINGALE (19) has stated that in general plants high in carbohydrates have relatively much of their assimilated nitrogen as protein, although the percentage of protein may be low, and has suggested that the duration of the light period is a factor in the utilization of carbohydrate for the conversion of inorganic nitrogen into protein form. A shift in the day length brings about a reworking of the metabolized products and an establishment of a new equilibrium between soluble and insoluble forms. Vegetative activity prevails when a high percentage of the metabolized nitrogen is present in a relatively soluble form, whereas flowering and reproduction are accompanied by an accumulation of carbohydrate and a decrease in soluble forms of nitrogen.

ADAMS (1, 2) has shown that plants exposed to a diminished light supply have a more rapid growth rate at first but ultimately the ones receiving the most light make the most growth. GILBERT (12, 13), in his work on the photoperiodic responses of *Xanthium*, found that under both high and low temperature conditions the ratios of total carbohydrate to total nitrogen and soluble carbohydrate to soluble nitrogen were distinctly ascending as flower primordia were formed.

ECKERSON (6) has found in Biloxi soy beans grown in an 8-hour day and a 16-hour night a decrease in nitrate reducing power of more than 95 per cent, and KNOTT (15) has found that the proper day length for the induction of flowering in *Cosmos* reduced the catalase activity in the stem tip to an extent of 109 per cent in 20 days. The length of the daily light period may also control the relative amounts of carotenoid pigments (18). CHIBNALL (5) found that at the time of flower bud formation asparagine is the most prominent product of the protein metabolism.

These investigations indicate that there are definite physiological changes accompanying flowering and reproduction. These changes have been induced entirely by the length of the daily light period as the external nutrient supply has remained constant. It is possible then to induce flowering entirely apart from any nutrient treatment. Flowering so induced is accompanied by a definite chemical change in the region of the apical primordium. The present study repre-

sents an attempt to determine more precisely the exact anatomical response of the primordium to the day length and the progressive chemical changes accompanying or preceding such anatomical change.

## Methods

Plants of *Cosmos sulphureus* Cav. var. Klondike were grown from seed in rich soil, in boxes 1×1×1 feet. They were kept under long day conditions by the use of 1000 watt Mazda lamps supplying an intensity of approximately 75 foot candles at the tops of the plants. Plants were grown under both 15- and 16-hour days, the lamps supplementing the particular number of daylight hours prevailing at the time of the experiment. Ordinary greenhouse temperatures prevailed. During the short day treatment the plants were kept at the same temperature as the long day controls. In the series run in May the night temperature varied between 65° and 70° F. while the day temperature rose to 90° or 100° F. for a short period during the afternoon. In the series grown in January the temperature remained nearly constant at 76° during the day and 65° F. at night.

Two methods of artificially producing short days were used. In May, when the temperatures were high at night, a screen of double thickness black sateen cloth was placed over the framework surrounding the plants at 4:30 P.M. and removed at 8:30 A.M., giving an 8-hour day. In winter the boxes were placed on a truck and moved into a dark chamber at 4:30 P.M. and out at 9:00 A.M., giving a $7\frac{1}{2}$-hour day.

Only relatively short periods of short day treatment are necessary to induce flowering in *Cosmos*. Accordingly separate groups of plants were given 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 consecutive short days respectively, and then exposed to long day treatment. Plants of each group were then examined periodically to determine the least number of short days necessary to induce flowering. The retarding effect of the subsequent long day treatment was determined by comparison with plants under short day treatment. A dissecting microscope proved the most useful means for determining the morphological response of the apical primordium. Material was also imbedded and prepared according to the usual paraffin method.

Microchemical as well as macrochemical analyses were made at various stages indicated as being significant by the detailed anatomical examination.

## Investigation

### Microchemical methods

Periodic examinations were made in order to follow the progress of the chemical changes occurring after the short day treatment was begun. Apical primordia of primary or lateral axes of purely vegetative long day plants were used in each case as a standard of comparison. The primordium itself and only the very closely associated stem tissue were used in the stem tip sample. The base of the stem was also examined to determine whether or not there was a change in this region accompanying the changes in the primordium. Comparable sections through stem tips of the long and short day plants were made by means of a sliding microtome in which both stems had been fastened by clamping them side by side in cotton, so that both were sectioned by the same stroke of the knife.

The microchemical tests found to be the most useful were:

Glutathione.—Based on the nitroprusside reaction of Hopkins, modified by Fink (7), this was used as follows: Heat sections in 10 per cent acetic acid to boiling and remove to three drops of saturated ammonium sulphate. Add two drops of 5 per cent sodium nitroprusside ($Na_2Fe(CN)_5 \cdot NO \cdot 2H_2O$). Allow to stand for 5 minutes. Add an excess of concentrated ammonium hydroxide (two or three drops). A pink to permanganate color indicates the presence of glutathione. The depth and duration of the color have been shown to indicate the amount present.

Arginine.—Based on the Sakaguchi (21) reaction which is specific for the free-guanidine group and therefore for arginine, this was used as follows: Place sections in one drop of 15 per cent NaOH. Add two drops of 0.15 per cent alpha naphthol. Add three or more drops of 0.5 per cent sodium hypochlorite. Red color on standing indicates arginine. It may be used quantitatively.

Histidine and tyrosin.—Based on Ehrlich's diazo reaction, this was used as follows: One drop of 0.5 per cent solution of sulphanilic acid in 2 per cent hydrochloric acid. Allow to react with one drop

of 0.5 per cent sodium nitrite for a minute or two. Add section to this and make the mixture alkaline with 10 per cent sodium carbonate. The presence of histidine is indicated by a deep red color. Tyrosin gives more of an orange tinge. The reaction can be employed quantitatively.

CONDENSATION REACTIONS WITH TRYPTOPHAN.—Treat sections with a 5 per cent solution of formalin for 15 minutes, then add strong hydrochloric acid to the mixture. A trace of an oxidizing agent such as ferric salts or nitrites speeds the reaction.

TYROSIN.—Based on the use of Millon's reagent as modified by BENSLEY (3).

TOTAL PROTEIN.—Based on various reactions; with $I_2KI$, ninhydrine, Millon's reagent, Sakaguchi reaction, etc.

FRUCTOSE.—Based on the reduction of Fehling's solution in the cold.

TOTAL REDUCING SUGAR.—Based on the reduction of Fehling's solution when heated.

Inulin, which is a storage product of *Cosmos*, seems to have a peculiar effect on the reduction of Fehling's solution by the reducing sugars, causing the formation of an amorphous copper complex which contains some crystals of $Cu_2O$ in its matrix. This difficulty was minimized by diluting the Fehling's solution (one part of mixed Fehling's solution to three parts of water). Crystals of $Cu_2O$ are also larger if this concentration is used.

## MACROCHEMICAL ANALYSES

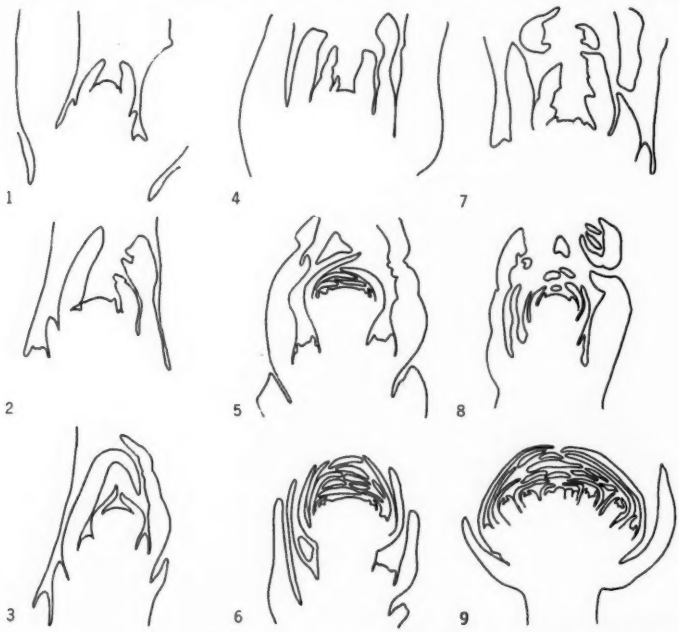
After the critical number of short days necessary to induce flowering was determined and the subsequent response of the primordia known, it was possible to bring the plants to any desired anatomical stage for macrochemical analysis. A period of ten short days was selected as an appropriate treatment of material to show the chemical differences between plants which had received the necessary stimulus to flower and those which would remain strictly vegetative under long day conditions.

The average height of the plants used for analyses was 12 inches. The stems were cut just above the ground and divided into two parts, the stem tip sample including the first inch of stem tip, and

the stem base sample including the remainder of the stem. Nitrogen fractionations and carbohydrate determinations were made on each sample.

## ANATOMICAL OBSERVATIONS

All plants do not react to short day treatment with exactly the same speed. Those which react the fastest may precede the slower

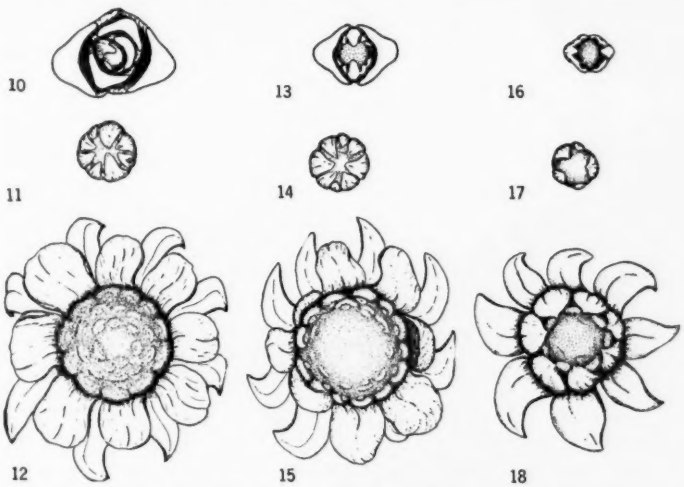



FIGS. 1–9.—Figs. 1–3, median sections through primordium of plants which have received seven 8-hour days followed by seven 16-hour days; figs. 4–6, seven 8-hour days followed by fourteen 16-hour days; figs. 7–9, seven 8-hour days followed by eighteen 16-hour days.

plants in the formation of flower buds by two or three days. Seven short days was the least number which produced an anatomical response, regardless of whether a $7\frac{1}{2}$-hour or an 8-hour daily light period was used. The plants which were exposed to an 8-hour daily light period in May responded 100 per cent within 9 days, whereas ten days were required for 100 per cent response under a $7\frac{1}{2}$-hour

daily light period in January. Figures 1–9 show the variations in the response of *Cosmos* primordia to seven 8-hour days in May.

It was thought that by reducing the length of the period of short day treatment to the absolute minimum necessary for the differentiation of flower primordia and then exposing the plants to the conditions of the long day, some expression of interphases (or "vegetative




FIGS. 10–18.—Fig. 10, view of primordium of a plant which has changed from an opposite to a spiral leaf arrangement; fig. 11, apical primordium from a plant which has received fourteen 7½-hour days; fig. 12, twenty-one 7½-hour days (the two sets of bracts are much further developed than the individual flower primordia); fig. 13, nine 7½-hour days (no bracts formed at this time); fig. 14, nine 7½-hour days followed by seven 15-hour days (first row of bracts just developing); fig. 15, nine 7½-hour days followed by fourteen 15-hour days; fig. 16, eight 7½-hour days; fig. 17, eight 7½-hour days followed by seven 15-hour days; fig. 18, eight 7½-hour days followed by fourteen 15-hour days.

flowers") would appear. In the group of plants given short day treatment in May, however, little tendency toward interphases appeared, save for the change from opposite to spiral leaf arrangement in 18 per cent of the plants (fig. 10).

The fact that all plants in the process of differentiating flower primordia gave strong evidence, after three weeks of subsequent long day treatment, of developing normal flowers, indicated that the light intensity or the daily light period might be further reduced and

a still closer approach to the minimum light requirement for flowering found. The same type of experiment was therefore made in January when the light intensity in Chicago is much lower. The daily light period was reduced to $7\frac{1}{2}$ hours. Groups of plants were exposed to 6, 7, 8, 9, and 10 short days respectively, after which they

TABLE I

RESPONSE OF COSMOS PLANTS TO DAILY LIGHT PERIOD OF 8 HOURS IN MAY, EXPRESSED IN PERCENTAGE OF NUMBER OF PLANTS USED

| No. of short days | Remaining vegetative | Changing to spiral | Forming flower primordia | No. of plants |
|---|---|---|---|---|
| 6 | 100 | | | 9 |
| 7 | 41 | 18 | 41 | 39 |
| 9 | | | 100 | 6 |

TABLE II

RESPONSE OF COSMOS PLANTS TO DAILY LIGHT PERIOD OF $7\frac{1}{2}$ HOURS IN JANUARY, EXPRESSED IN PERCENTAGE OF NUMBER OF PLANTS USED

| No. of short days | Remaining vegetative | Changing to spiral | Forming flower primordia | No. of plants |
|---|---|---|---|---|
| 6 | 100 | | | 10 |
| 7 | 67 | 20 | 13 | 15 |
| 8 | 40 | 11 | 49 | 26 |
| 9 | 20 | 27 | 53 | 15 |
| 10 | | | 100 | 10 |

were removed to conditions of the long day. Electric lights (1000 watt Mazda lamps, giving approximately 75 foot-candles intensity at the top of the plants) were used to supplement the normal day length, giving a 15-hour day. The results obtained are shown in tables I and II.

The differences in the rates of development of flowers by plants which had received 7, 8, and 9 short days are shown in figures 10–18. The retarding effect on floral development produced by long days following the short day treatment may be seen by comparing plants

that were shifted to long day conditions with those that were maintained continuously under short day illumination (figs. 11, 12).

A third group of plants which had attained a height of 12–16 inches was divided into three lots. The first lot received eight $7\frac{1}{2}$-hour days, the second, nine $7\frac{1}{2}$-hour days, and the third, ten $7\frac{1}{2}$-hour days, each being immediately placed under conditions of the long day after the treatment. These plants were allowed to grow in the long day conditions (15 hours of light per day) for three months. At the end of this period a very decided difference in the floral development was found.

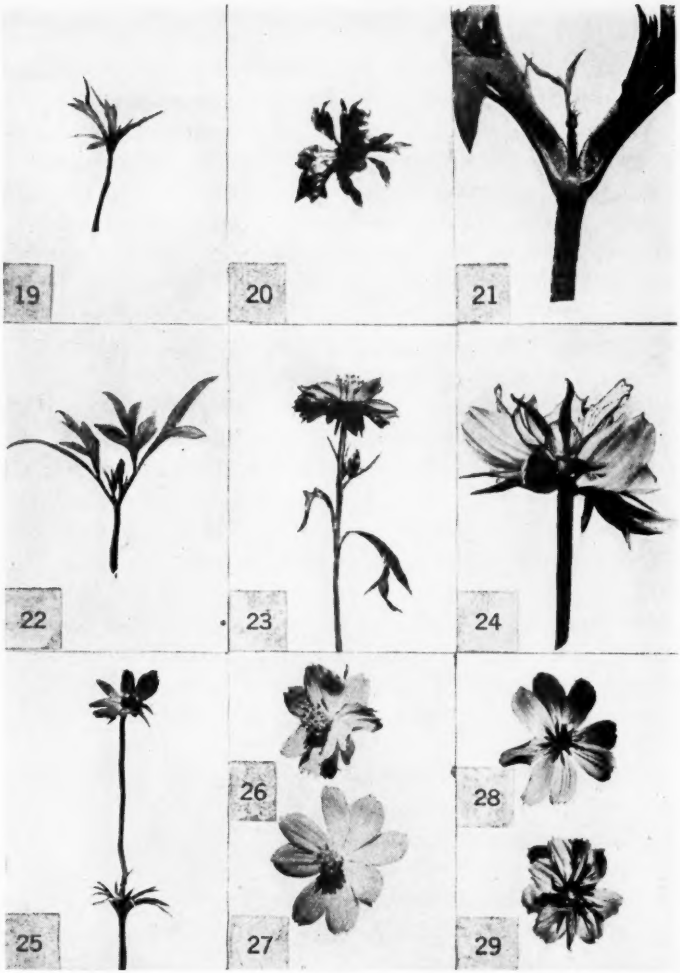
The normal *Cosmos* flower has two rows of involucral bracts, usually eight in each row. The outer set is foliaceous and spreading while the inner set is broader and nearly membranous. The ray flowers, usually eight in number, appear in the axils of the inner set of involucral bracts. Each disk flower is borne in the axil of a bractlet on the determinate composite head. The bracts ordinarily remain shorter than the disk flowers and are membranous in nature.

The first flowers to open in the foregoing group were those which had received ten short days. Two months of long days were required before the first flowers opened. The control plants, which were kept under continuous short day conditions, began to blossom in six weeks. The following differences in the flowers were noticed (figs. 26–29):

Continuous short day treatment until flowering ($7\frac{1}{2}$-hour days)
- Length of first row of bracts..... 5 mm. (fig. 28)
- Length of second row of bracts..... 9 mm.
- No. of ray flowers....8; length..... 20 mm. (fig. 27)

Ten short days followed by two months of long days ($7\frac{1}{2}$- and 15-hour days)
- Length of first row of bracts..... 16 mm. (fig. 29)
- Length of second row of bracts..... 12 mm.
- No. of ray flowers....14; length..... 20 mm. (fig. 26)

Figures 19 to 25 show the types of variations from normal flowers which were found. There are four important points concerned with their development. First, the primordium initiates a flower and becomes determinate, but owing to the influence of the long days the bracts and bractlets elongate and become foliaceous and the flowers




FIGS. 19–29.—Figs. 19, 20, apex of stems from plants which have received eight 7½-hour days followed by sixty 15-hour days (bracts have become foliaceous and flowers are suppressed); fig. 21, eight 7½-hour days followed by sixty 15-hour days (flower bud suppressed); figs. 22–24, developing flowers from plants which have received nine 7½-hour days followed by sixty-five 15-hour days (division of flower primordium shown); fig. 25, nine 7½-hour days followed by sixty-five 15-hour days (abnormal elongation of stem between bracts and flowers shown); figs. 26, 29, front and rear views of flower from a plant which has received ten 7½-hour days followed by sixty 15-hour days; figs. 27, 28, front and rear views of flower from a plant which has received continuous treatment under 7½-hour daily light period.

fail to develop beyond mere appendages in their axils (figs. 19, 20). Second, if the tendency to flower is a little stronger the flower head may develop to a diameter of about 3 mm. and then become permanently suppressed. The axillary buds immediately below become active vegetatively and develop into new shoots which assume apical dominance (fig. 21). Third, if the tendency to flower is still stronger the flower head may develop and produce a mature flower but there is a marked tendency for the primordium to divide, resulting in two flower heads. The division may result in two flower stalks of equal length, or the division may not result in two flower stalks but in the appearance of two flower heads on one stalk (figs. 23, 24). Fourth, when there is considerable retardation of floral development by the long days, an internode of considerable length may develop between the involucral bracts and the bractlets of the floral head (fig. 25); or the elongation of the stem in the region of the involucral bracts may result in their separation longitudinally, leaving them spirally arranged over a distance of 1–2 cm. of the stem immediately below the flower head.

## Microchemical results

Vigorously vegetative plants were taken from a 15-hour day and given a $7\frac{1}{2}$-hour day. Thereafter examinations were made daily at 9:00 A.M. and 7:00 P.M. for a period of 14 days. Long day strictly vegetative plants were used as a standard of comparison at each examination.

From the morning and evening examination of the primordia a diurnal fluctuation in the glutathione content was discovered. It was found that in the morning the primordia of the short day plants contained a considerably greater quantity of glutathione than those of the plants which had received a long day. After both plants had been exposed to a period of light lasting until evening, however, the glutathione content of the primordia was approximately the same. This nightly fluctuation of glutathione in the short day plants continued until about the seventh day, when it became and remained permanently greater than in the vegetative plants. This observation was extended to the fourteenth day, at which time the flower primordium is well developed.

CARBOHYDRATES.—A marked increase in reducing sugars appeared in the stem tips of the short day plants by the fourth day of treatment. From that time on to the fourteenth day, when observations were discontinued, there was a progressive increase in the amount present. The accumulation of reducing sugar appeared before there was any marked increase in the protein content of the stem tip.

NITROGENOUS FRACTIONS.—There was no marked increase in the total amount of organic nitrogenous material in the stem tips of the short day plants during the fourteen days of observation. The relative amount of soluble and insoluble organic nitrogenous products, however, changed markedly. In the stem tips of the plants which had received short day treatment there was an accumulation of proteinaceous material beginning about the fifth or sixth day and becoming marked by the seventh day, from which time it continued to increase. The protoplasm of the cells increased in density.

As the reactions employed for the detection of amino acids are given by both the free amino acids and proteins, there was some difficulty in the determination of the relative amounts of protein and soluble organic nitrogen. This difficulty was best overcome by denaturing the proteins before the color reactions were employed. The proteinaceous material then gave a much better test with Millon's reagent and ninhydrine reactions than undenatured material. With reasonable care in the preparation of the sections, and the comparison of one section with another, there was little difficulty in demonstrating the greater accumulation of complex protein material in the stem tips of the short day plants than in the vegetative tips of long day plants.

## MACROCHEMICAL RESULTS

The tenth day after the beginning of the short day treatment was selected as the best time for the collection of samples for analysis, for by that time all plants would have received the necessary stimulus to flower yet there would have been no marked anatomical change. Purely vegetative long day plants were used as the controls.

Carbohydrate analyses were made by mincing the fresh tissue and extracting with hot 95 per cent alcohol. The soluble reducing sugars were determined therefrom by the Bertrand volumetric method.

The insoluble residue from the extraction was boiled in 2.5 per cent hydrochloric acid for three hours and the hydrolyzable carbohydrates determined by the same method. Sucrose was not determined.

Fresh tissue only was used for nitrogenous fractionations. Extractions were made with water according to the methods of CHIBNALL (4) and determinations were made according to the usual methods (20). All samples were made in duplicate and checks were run on each sample. All differences greater than 2 per cent are significant except for ammonium, nitrate, and amide nitrogen. Here the variations between the several extractions differ by 15 per cent. The difficulties involved in sampling and extracting such small aliquots make the variability of results high.

TABLE III

ANALYSIS OF PLANTS HAVING RECEIVED 10 SHORT DAYS, AND LONG DAY VEGETATIVE PLANTS, EXPRESSED IN PERCENTAGE OF GREEN MATTER

| | SHORT DAY STEM TIPS | LONG DAY STEM TIPS | PERCENTAGE DIFFERENCE | SHORT DAY STEM BASES | LONG DAY STEM BASES | PERCENTAGE DIFFERENCE | SHORT DAY WHOLE STEM | LONG DAY WHOLE STEM | PERCENTAGE DIFFERENCE | PERCENTAGE VARIATION OF SAMPLES |
|---|---|---|---|---|---|---|---|---|---|---|
| $NH_3$ | 0.0032 | 0.0128 | 75.0 | 0.0047 | 0.0095 | 50.5 | 0.0079 | .0223 | 64.6 | 15 |
| Amide | 0.0378 | .0284 | 24.7 | .0140 | 0.0182 | 23.1 | .0518 | .0466 | 10.0 | 15 |
| $NO_3$ | .0061 | .0061 | 00.0 | .0054 | .0090 | 40.0 | .0115 | .0151 | 23.8 | 15 |
| Soluble organic N | .1247 | .1328 | 6.1 | .1497 | .1366 | 8.7 | 0.2726 | .2694 | 1.2 | 2 |
| Protein N | .2315 | .2155 | 6.9 | .0820 | .1080 | 24.1 | .3125 | .3235 | 3.4 | 2 |
| Total organic N | .3562 | .3483 | 2.2 | .2299 | 0.2446 | 6.0 | .5861 | .5929 | 1.1 | 2 |
| Hydrolyzable carbohydrate | 1.515 | 1.515 | 0.0 | 1.380 | 1.650 | 16.4 | 2.895 | 3.165 | 8.5 | 2 |
| Reducing carbohydrate | 0.5360 | 0.4640 | 13.4 | 0.4800 | 0.5360 | 10.4 | 1.016 | 1.000 | 1.6 | 2 |
| Dry weight | 8.980 | 9.310 | 3.5 | 8.000 | 9.550 | 16.2 | 8.490 | 9.430 | 10.0 | 2 |

## Discussion

*Cosmos* is affected so strongly by a short daily light period that it will flower and set seed after being exposed to only ten $7\frac{1}{2}$-hour

days. When flower primordia are initiated by a fewer number of short days the tendency to flower has not always been so definitely established that it may not be reversed by subsequent treatment with long days, producing "vegetative flowers." The short day treatment obviously has some marked effects on the chemical processes associated with flowering. The chemical changes accompanying the anatomical change agree closely with other observations concerning flowering and reproduction, especially in regard to the carbohydrate-nitrogen relation (**16**).

The composition of the whole plant does not change markedly, except that there is a decrease in dry weight of 10 per cent during the ten short days (**17**). This can be accounted for mainly by the decrease in polysaccharides of 8.5 per cent and a slight decrease in protein. As the amount of materials manufactured during the shortened days is markedly less, it seems important to consider the states of the metabolized materials as they are related to the daily light period. Under the shortened light exposure there is a marked tendency toward the concentration of soluble carbohydrate and complex protein in the stem tip at the expense of the polysaccharides and proteins in the base of the stem. There are differences in asparagine and ammonia and undoubtedly in many other cellular constituents, but a more complete study of the composition of the proteins is needed in order to give them meaning. The important point is that it requires approximately seven short days for the stem tip to acquire a definite and marked accumulation of reducing sugars, and a change in the state of the proteins toward a much more complex and difficultly soluble form. Any treatment involving fewer short days than is necessary to bring about this change is without effect in inducing flowering, but a few added days of treatment, once the accumulation has begun, giving time enough for the accentuation of the changes, insured 100 per cent conversion of the vegetative primordia to the flowering condition.

The initiation of a flower primordium is associated with a definite metabolic state of the stem tip. The changes in the metabolism of the vegetative plant which result in flowering due to photoperiodic treatment include a probable proteolysis of the proteinaceous material and the hydrolysis of polysaccharides in the base of the stem

and their removal to the stem tip. The photoperiod influences (1) the amount of material which the plant is able to conserve, (2) the use to which it will be put, and (3), the quality of the proteins and carbohydrates in the various regions.

The amount and kind of carbohydrate and protein as determined in these experiments, and the diurnal fluctuation in the amount of glutathione, seem to be factors intimately connected with the photoperiodic treatment which has a definite correlation with the morphological change of the primordium from vegetative to reproductive. Any conclusions as to the meaning of the fluctuation of glutathione content with the change in the daily light period cannot be made until more is known concerning the function of glutathione. In the light of certain recent findings by HAMMETT (14), it might seem that glutathione is a factor which modifies the metabolism of proteins. At any rate it seems evident that both carbohydrates and proteins are influenced by the photoperiod, but whether it is directly or indirectly, through the action of some intermediate compound, has not been determined.

## Summary

1. The plants of *Cosmos sulphureus* Cav. var. Klondike reacting most readily to short day treatment required seven short days for the initiation of a flower primordium. The slowest plants required ten days. When the absolute minimum treatment necessary to induce flowering was given, followed by long days, there was a marked tendency toward the production of interphases between normal flowers and vegetative shoots.

2. The change of the primordium from foliar to floral was accompanied by a marked accumulation of carbohydrate and protein in the stem tip. There is a probable proteolysis and a decrease in protein in the base of the stem at the time of the conversion of the primordium to the flowering state. There is also hydrolysis of carbohydrates in the base of the stem and their removal to the stem tip at the same time.

3. The shortened light period caused a decrease of 1 per cent per day of the total materials stored.

4. There was a diurnal fluctuation of glutathione in the stem tip during the first seven days of short day treatment, but from the

time of the actual anatomical shift of the primordium from vegetative to flowering the total glutathione content remained permanently higher.

5. Asparagine was somewhat higher and ammonium somewhat lower in the stem tip at the time of flower bud formation.

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# PENNSYLVANIAN FLORA OF ILLINOIS AS REVEALED IN COAL BALLS. II.

ROY GRAHAM

(WITH TWENTY-FOUR FIGURES)

The following descriptions are based on coal ball material from the Calhoun coal mine, Richland County, Illinois, horizon McLeansboro (Upper Conemaugh). The specimens which form the basis of these descriptions are in the writer's collection, but duplicate sections of most of the material are in the collections of the botany departments of the universities of Cambridge and Chicago.

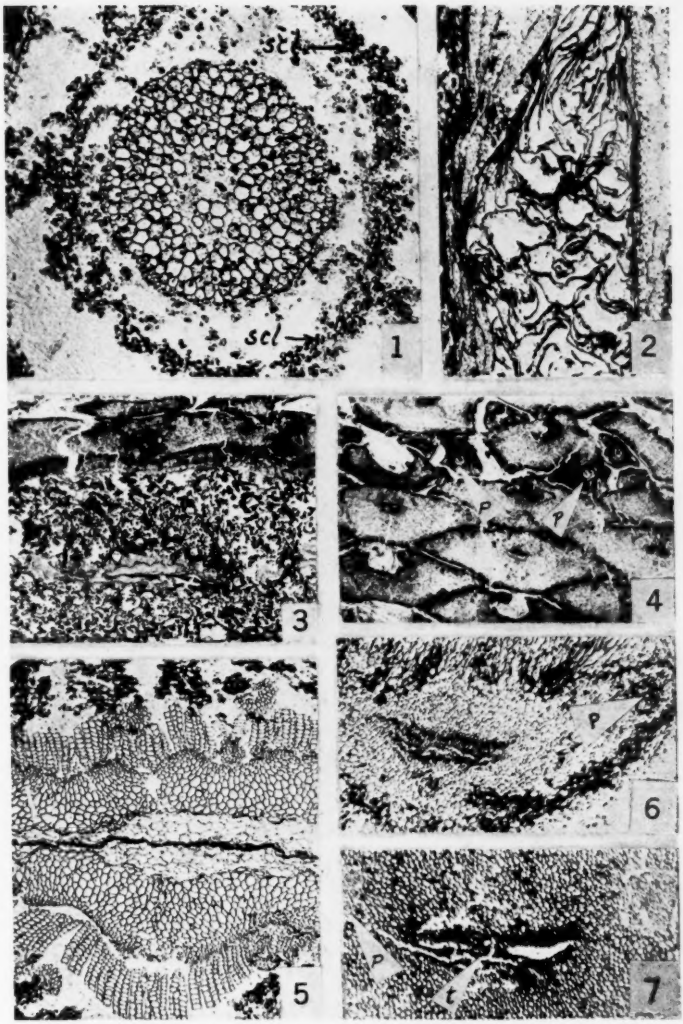
*Lepidodendron* cf. *L. selaginoides* Sternb.

A number of small branches and a number of closely associated leaves were found in two coal balls. The largest branch measures 1.2 cm. in diameter and has a stele 0.2 cm. in diameter. The smooth outline of the corona and the presence of a few tracheids in the pith suggest a comparison with *L. selaginoides* (**9**), or with *L. hickii* Wats. (**14**). There are a greater number of tracheids in the pith of *L. selaginoides* than in *L. hickii*, but in these small specimens the pith area is too limited to make certain identification on the basis of this character. The presence of a ring of sclerenchyma (*scl*, fig. 1) in the inner cortex suggests identification with *L. selaginoides*.

*Lepidostrobus* sp.

A number of small lycopodiaceous strobili occur in one coal ball. The strobili are of two kinds, one containing megaspores and the other microspores. Detailed description is impossible on account of poor preservation.

Several longitudinal tangential sections of the tip of one strobilus give the best clue to the structure. The most nearly radial section (fig. 2) just grazed the cortex of the central axis. The portion preserved measured 15 mm. long by 3.5 mm. in diameter. The sporophylls stand out almost at right angles to the axis; they are very thick at the base and are joined to the axis by a short stout attach-




FIGS. 1–7.—*Lepidodendron* cf. *L. selaginoides*, transverse section of stele and encircling zone of sclerenchyma (*scl*); ×20. Fig. 2, *Lepidostrobus* sp., longitudinal section of tip of strobilus; ×8. Figs. 3–7, *Sigillaria approximata:* 3, transverse section of stem ×2; 4, longitudinal tangential section through leaf bases and verticil of peduncles (*p*) ×2; 5, transverse section of portion of stele ×20; 6, longitudinal tangential section in region of secondary cortex showing leaf trace (*t*) surrounded by parenchyma which is continuous with parichnos (*p*) ×20; 7, a more superficial section of the same, exterior to the secondary cortex (*t*, leaf trace; *p*, parichnos); ×20.

ment; there is no definite pedicel. The sporophyll is broad and winged, and its leaflike lamina does not have a downward projection. The sporangia are only slightly elongated in the radial direction and are attached to the upper surface of the sporophyll by a pad of transfusion tissue. The sporangium wall consists of a single prismatic layer. Only fragments of thick walled megaspores remain.

Transverse sections measuring about 20 by 4 mm. are too badly crushed and disorganized to show the shape and arrangement of the sporophylls. The axial stele, 0.38 mm. in diameter, has a small pith and prominent peripheral protoxylem points. The flattened megaspores had an original diameter of about 1 mm.

Disorganized microstrobili, agreeing with the megastrobili in size, and in the character of the axial stele and of the sporangium wall, contain numerous microspores 50 $\mu$ in diameter.

Whether these two kinds of strobili belong to the same species or not must await the discovery of more complete material. The lax nature of the cone and the absence of a downward projection of the lamina distinguish this from previously described species.

*Sigillaria approximata* Font. & White (**8**)

DIAGNOSIS.—Clathrarian type. Leaf bases tangentially elongated; vascular bundles and parichnos strands in upper half of scar. Primary xylem a complete ring inclosing a large pith. Corona markedly sinuate. Secondary xylem developed. Leaf traces without secondary xylem, at first mesarch, becoming tangentially elongated and with complete or nearly complete reduction of the centrifugal xylem, finally dividing into a double bundle in the leaf base. Middle cortex containing nests of sclerenchyma and sclerenchymatous sheaths about the leaf traces. Parichnos strands passing directly inward where they merge with the parenchyma accompanying the leaf traces. Small triangular peduncle scars occurring in verticils. Xylem strand of peduncle with peripheral protoxylem and without secondary thickening.

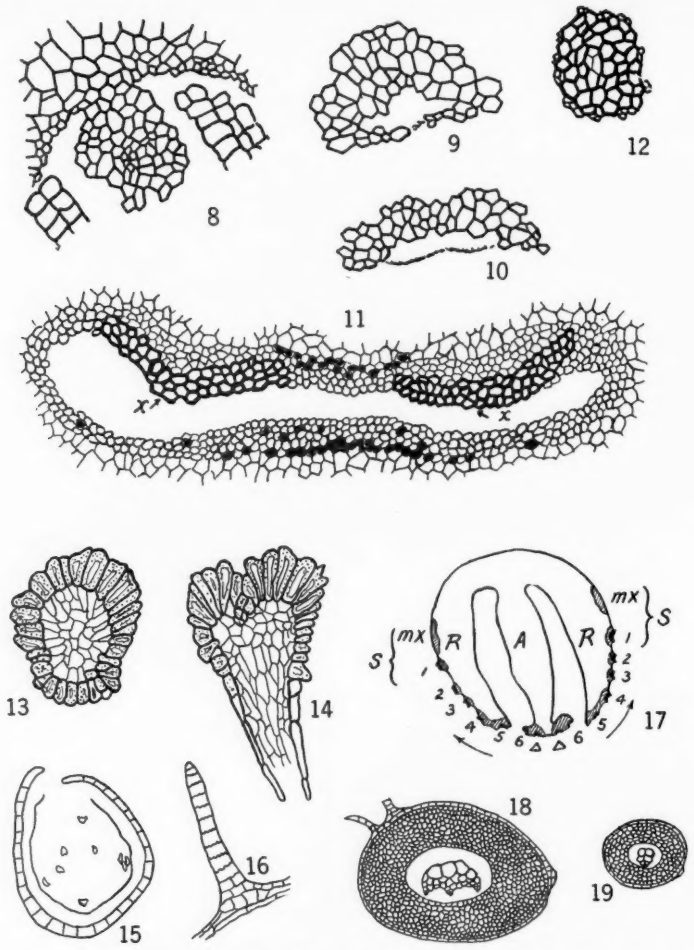
The stem is much flattened and slightly crushed, but portions of all tissues except the delicate ones immediately outside the xylem are clearly preserved (fig. 3). The specimen is about 25 cm. in length and its diameter exceeds 5.6 cm. The diameter of the stele is 1.8 cm.

Tangential sections through the leaf bases show that the stem is of the Clathrarian type (fig. 4). There are verticils of triangular organs (*p*, fig. 4) which are believed to be the scars where the peduncles of strobili were attached. These peduncle scars occur between longitudinal series of leaf bases whose shape they modify, but they do not appear to disturb the phyllotaxy. The shape and arrangement of the leaves and peduncles agree with ZEILLER'S (**15**) figure of *Sigillaria approximata* and with FONTAINE and WHITE'S (**8**) original description of impressions of this species.

The ring of primary xylem is continuous and is from 0.5 to 0.8 mm. in thickness. The outer margin is deeply and regularly undulate. Most of the large pith is destroyed by crushing (fig. 5). The secondary xylem forms a zone 0.3–0.6 mm. wide. The structure of both primary and secondary xylem agrees in all respects with that of *Sigillaria elegans* (**10**).

The leaf traces arise from the periphery of the primary xylem and invariably at the base of the furrows. When the leaf trace is about to separate from the primary xylem it consists of a circular group of from 25 to 50 tracheids (fig. 8). The bundle is mesarch, the smallest elements (protoxylem) being placed eccentrically toward the outer margin of the group. By the time the leaf trace emerges from the secondary xylem, it has elongated in the tangential direction (fig. 9). There is no secondary xylem in the leaf trace. The protoxylem is not actually seen at this level, but it is believed to have occupied part of the vacant space which is found in the center of the bundle. The bundle thus retains its mesarch structure but the centrifugal xylem is reduced. Farther up, the leaf trace becomes more and more tangentially elongated, with still more reduction of the centrifugal xylem, which may die out completely (fig. 10). The leaf trace passes through the secondary cortex in a horizontal direction, accompanied by an encircling zone of parenchyma (fig. 6). Within the leaf base it divides into a double bundle (*xx*, fig. 11).

No tissue is found in organic continuity with the exterior of the secondary xylem, the cambium and phloem not being preserved. The inner cortex consists of parenchyma, of which only fragments are preserved, and of numerous isodiametric sclerenchyma cells which occur both as scattered groups throughout the parenchyma and as sheaths surrounding the leaf traces.




FIGS. 8–19.—Figs. 8–12, *Sigillaria approximata*, all ×75; 8, leaf trace being given off from border of primary xylem; 9, same in inner part of cortex; 10, same at outer margin of middle cortex; 11, same within leaf base (note double vascular bundle (*xx*) and gap below bundle caused by disintegration of delicate tissue); 12, bundle of peduncle. Figs. 13–16, *Botryopteris americana*, all ×30: 13, 14, transverse and longitudinal sections of sterile sporangium; 15, sporangium with spores; 16, equisetiform hair. Fig. 17, diagram of branching of *B. forensis* (after BERTRAND) (*A*, central vascular band; *RR*, receptive pieces; ΔΔ, fundamental poles; 1 to 6, strands of protoxylem detached from poles and each destined for a secondary rachis; *SS*, masses destined for a secondary rachis). Each branch stele is formed by the union of a mass (*mx*) and a strand of protoxylem. Figs. 18, 19, *Botryopteris* cf. *B. hirsuta* or *B. ramosa*, transverse sections of petioles ×12: 18, petiole with triarch bundle; 19, smaller petiole with monarch bundle.

The general form of the leaf bases has already been described. Occurring in the upper third of the leaf are the tangentially elongated vascular bundle and the two parichnos strands which are lateral to and slightly above the bundle (fig. 7). No ligule was recognized in any of the sections. Immediately below the vascular bundle is a space due to the disintegration of delicate tissues (fig. 11). The parichnos consists of delicate parenchyma, and its general appearance is that of aerenchyma. There is no differentiation into two zones as in *S. spinulosa*, but cells with dark contents are often found surrounding the parichnos. Internally the parichnos strands do not unite beneath the vascular bundle as in *S. spinulosa*, nor do they broaden out and finally surround the bundle as in *S. scutellata*, but continue unchanged into the inner part of the leaf base. In this region and in the outer part of the secondary cortex the parichnos changes its character and gradually merges with and becomes indistinguishable from the parenchyma which surrounds the leaf trace on its outward course through the secondary cortex (fig. 6).

The peduncle scars are roughly triangular in shape (*p*, fig. 4) and are much smaller than the leaf bases. They agree in general structure with those of *S. elegans*. The vascular bundle of the peduncle is roughly circular in section (fig. 12). The tracheids have very thick walls. Preservation is imperfect but the protoxylem is evidently peripheral. No leaf traces are given off by the peduncle bundle at this level. The cortex exhibits no special features. The tissues, in particular the conducting elements, are not so well preserved as are those of the leaf bases. This suggests that the strobili had fallen long before fossilization.

In external characters *S. approximata* is distinct from *S. elegans*, but the anatomy of their tissues is almost identical and this suggests a close relationship.

*Sigillaria approximata* is a Sub-Sigillarian species which exhibits characters formerly regarded as characteristic of the Eu-Sigillariae (1), namely, the possession of a continuous ring of primary xylem, monoxylic leaf traces (absence of secondary wood in the leaf trace), and a double leaf trace in the region of the leaf bases. This lends support to the doubts which have been expressed regarding the value of the sections Eu-Sigillariae and Sub-Sigillariae as indicative of actual differences (13).

*Mazocarpon* cf. *Mazocarpon shorense* Bens.

Several isolated megaspores, more or less reniform in section, were found. One specimen was still imbedded in sterile tissue (*sp*, fig. 20) still inclosed by a portion of the sporangial wall. Apart from the absence of any projections from the convex surface of the spore, these specimens are similar to and may be identified with *Mazocarpon shorense* (3).

*Botryopteris* **americana** sp. nov.

DIAGNOSIS.—Stele of petiole U-shaped in cross section, the three long xylem arms projecting abaxially. Pinna traces given off alternately from the terminal portion of the lateral xylem arms. Absence of fibrous sclerotic elements surrounding the xylem on its convex side and of gum canals in the cortex. The petiole bears equisetiform hairs. Sporangia and peculiar sterile sporangia as in *B. forensis*.

Sections of branching petioles were obtained. The larger of the petioles (fig. 21) has a diameter of 5 mm. Its structure is very similar to that of *B. forensis* Renault (11, 12). The following differences were noted: the absence of a sheath of thick walled cells, almost fibrous in nature, surrounding the xylem on its convex side; and the absence of gum canals in the cortex.

Certain isolated structures and organs, occurring abundantly in the same coal ball as the petioles, may be ascribed with certainty to *Botryopteris americana* on account of their agreement with similar structures borne by *B. forensis*. These are specialized sterile sporangia (figs. 13, 14), sporangia with spores (fig. 15), and equisetiform hairs (fig. 16).

At the end of the central xylem arm of the petiole are borne two crescentic groups of protoxylem, the "fundamental poles" of BERTRAND (6). According to BERTRAND and CORNAILLE (4), these give rise to groups of protoxylem which pass laterally to the tip of the lateral xylem arms. These protoxylem groups shift their position along the external face toward the base of the arm. Owing to poor preservation it was not possible to see this in the writer's specimens. BERTRAND'S (6) diagram of the course of the protoxylem bundles in branch formation is reproduced (fig. 17). In the writer's sections

there are two cases of branching and in both the method of branch formation differs from that shown by BERTRAND. The terminal portion of the xylem arm, bearing all the protoxylem groups, separates to form the lateral branch (fig. 21) instead of only a small portion of xylem from the side of the arm as shown by BERTRAND. The mode of origin of the pinna trace is thus similar to that of *B. antiqua* (**2**).

*Botryopteris* cf. *B. ramosa* Will. & *B. hirsuta* Will.

There are several small petioles in a poor state of preservation. The largest has a diameter of about 1.5 mm. The vascular strand is crescentic, with three small projections on the concave side (fig. 18). Smaller petioles have a monarch bundle (fig. 19). Specific identification is not possible, but the petioles agree in structure with those of *B. ramosa* or *B. hirsuta*.

*Anachoropteris* **clavata** sp. nov.

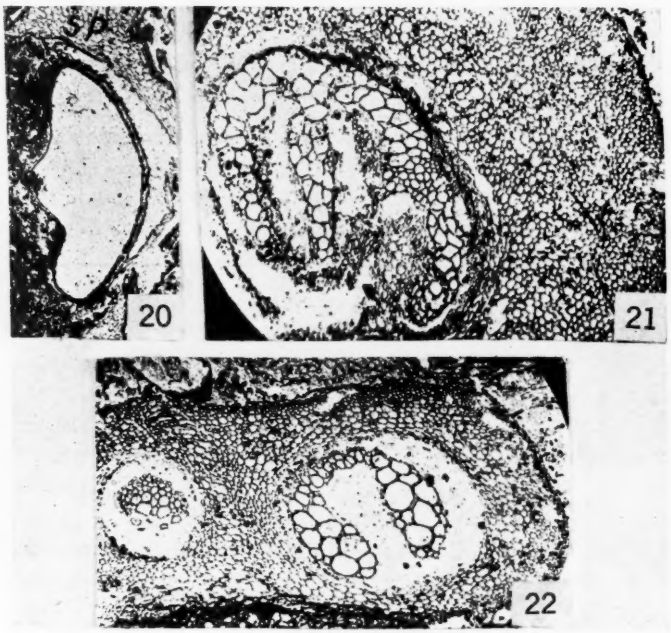
DIAGNOSIS.—Stele of petiole horseshoe-shaped in cross section, the arms pointing abaxially and expanding distally. Protoxylem groups occurring as projecting points along the adaxial side of the stele. Cortex not projecting between the xylem arms. Pinna traces given off alternately from the external face of the xylem arms.

Transverse and longitudinal sections of a branching petiole were prepared. In transverse section the unbranched petiole measures 2.8 by 1.7 mm. The stele is a large U-shaped bundle with the arms of the U much swollen and club-shaped (fig. 22), from which character the species derives its name. From the outline of the petiole it appears that the xylem arms pointed away from the stem.

The largest tracheids are in the arms of the U and the smaller tracheids are found toward the base. There are about six projecting protoxylem points along the convex side of the base, the petiole thus being endarch. The protoxylem occurs as distinct projecting points and not as projecting crescentic cusps as in *A. decaisnii* (**5**). The tracheids of the protoxylem points have close spiral or scalariform pitting. The metaxylem elements have crowded elliptical bordered pits arranged in spiral sequence on all walls.

The cortex consists of moderately thick walled cells elongated parallel to the axis. The cells become progressively longer and nar-

rower toward the exterior. There is no tongue of cortex projecting between the xylem arms as in previously described species of the genus. All the cortical cells have transverse end walls. Sclerenchyma and gum canals are absent. The epidermis bears short stout multicellular hairs.




FIGS. 20–22.—Fig. 20, *Mazocarpon* sp., megaspore and portion of sporangium (*sp*); ×20. Fig. 21, *Botryopteris americana*, transverse section of petiole. Note distal portion of lateral arm beginning to separate off to form a branch stele; ×20. Fig. 22, *Anachoropteris clavata*, transverse section of branching petiole; ×20.

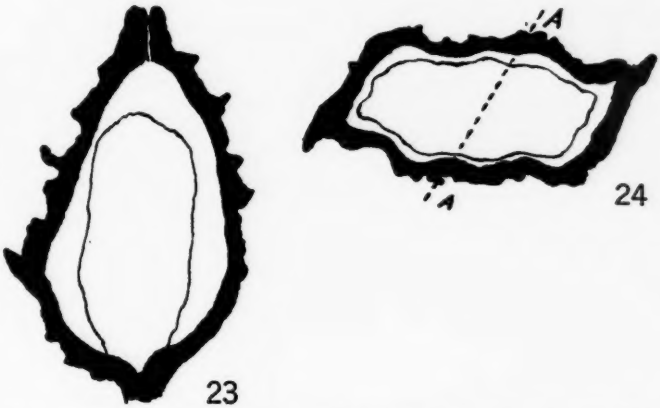
The method of branch formation is the same as in *A. decaisnii*.

*Anachoropteris clavata* is distinct from any of the previously described species of that genus, in possessing protoxylem points instead of crescentic cusps, in the much expanded xylem arms, and in the absence of a tongue of cortex projecting between the xylem arms.

*Cardiocarpus* **spinatus** sp. nov.

DIAGNOSIS.—Platyspermic seeds with double vascular system. Outer vascular system separates from the chalazal bundle before the latter pierces the sclerotesta. Sclerotesta thick, with spiny outgrowths. Size about 6.5 mm. long by 8 mm. wide by 3.5 mm. thick.

Longitudinal sections were made of two seeds, and transverse sections of the half of one (figs. 23, 24). The seeds are decidedly platyspermic. One measures 6.5 mm. long by 8 mm. wide by 3.5 mm.




Figs. 23, 24.—*Cardiocarpus spinatus:* Fig. 23, longitudinal section; ×8. Fig. 24, transverse section (*A–A*, position of section illustrated in fig. 23); ×8.

thick; the other is slightly larger. The thickest part of the seed is about one-third the distance from the base to the summit. The base of the seed is rounded; the apex is produced into a short and narrow micropylar canal. In transverse section the seed is lenticular, and bears two ribs in its principal plane. These descriptions and measurements refer to the exterior of the sclerotesta, since the outer fleshy layer, the sarcotesta, has been almost completely destroyed.

The moderately thick sclerotesta consists of two layers of tissue, an inner layer of moderately thick walled cells elongated in the direction of the axis of the seed, and an outer layer of much thicker walled, more or less isodiametric cells. This outer layer is of vari-

able thickness and has spines at its outer surface. These projections may not have been visible, being originally imbedded in the fleshy portion of the seed.

The nucellus is very poorly preserved, only the superficial layer of large polygonal platelike cells remaining. No pollen chamber is discernible, possibly because of the poor state of preservation, nor was it possible to determine how much of the nucellus was free from the integument. The megaspore membrane is preserved.

The vascular system of the seed is double, consisting of an outer system within the sarcotesta and an inner system within the nucellus. The outer system consists of a bundle of delicate tracheids running longitudinally the full length of the seed along the outer margin of each of the two sclerotestal ribs. As far as could be determined from the unfavorable planes of section, this outer system separates from the chalazal bundle before the latter pierces the sclerotesta. The inner system consists of the continuation of the main bundle which pierces the sclerotesta at the chalaza and expands into a plate of transfusion tissue in close contact with the megaspore membrane within the base of the nucellus. There is no indication of the inner vascular system extending up the sides of the nucellus.

The platyspermic form of this seed, and its double vascular system (the outer set of bundles are given off from the chalazal bundle before the latter pierces the sclerotesta) are characters which place it in the genus *Cardiocarpus*. It is a well marked species and is less than half the size of any previously described. In the possession of a thick stony testa it resembles *C. sclerotesta* Br. (7), but differs from that species in the less sclerotic nature of the inner layers of the integument and in the spiny outgrowths of the exterior of the sclerotesta.

*Calamites* sp.

Roots and rootlets.

*Sphenophyllum plurifoliatum* Will. & Scott

Stem.

*Psaronius* sp.

A badly crushed stem, polystichous type, with numerous steles and bands of sclerenchyma. It is too much crushed to permit of specific determination.

*Heterangium tiliaeoides* Will.

Several stems were identified.

*Medullosaceae*

A portion of a stem, obviously belonging to the family Medullosaceae, but too incomplete for even generic determination, and four species of petioles of *Myeloxylon*, were noted.

## Summary

1. This paper records the occurrence of a number of plants found as petrifacts from Calhoun coal mine, Richland County, Illinois. The plants belong to the genera *Calamites*, *Sphenophyllum*, *Lepidodendron*, *Lepidostrobus*, *Sigillaria*, *Mazocarpon*, *Botryopteris*, *Anachoropteris*, *Psaronius*, *Heterangium*, *Myeloxylon*, and *Cardiocarpus*.

2. It appears that there are no constant anatomical distinctions between the Eu-Sigillariae and the Sub-Sigillariae.

3. The branching of petioles of *Botryopteris americana* is described.

This study was made possible through the grant of a fellowship from the National Research Council, to which gratitude is expressed. The writer is much indebted to Professor A. C. SEWARD under whose direction the study was carried out, and to Dr. A. C. NOÉ who kindly supplied the material, collected under the auspices of the Illinois State Geological Survey.

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# CYTOLOGICAL STUDIES IN THE CHENOPODIACEAE

## I. MICROSPOROGENESIS AND POLLEN DEVELOPMENT

GEORGE OLDS COOPER

(WITH FORTY-TWO FIGURES)

### Introduction

The purpose of this investigation was to describe in detail the male gametes and to determine the chromosome numbers in certain members of the Chenopodiaceae. Most observers working with angiosperms have mentioned male nuclei as occurring in the microgametophyte, but in only a few instances have the male gametes been described as distinct cells. TUSCHNJAKOWA shows them to be cells in *Spinacea oleracea* (**10**, fig. 20*a*). The presence of such male cells, aside from those in the Chenopodiaceae, has been observed by FRYE (**5**) and FINN (**4**) in certain members of the Asclepiadaceae, by WYLIE (**12**) in *Elodea canadensis* and (**13**) in *Vallisneria spiralis*, by SHATTUCK (**8**) in *Ulmus americana*, by PODDUBNAJA-ARNOLDI (**6**) in *Scabiosa purpurea*, and by COOPER (**3**) in *Portulaca oleracea*. STOMPS (**9**), WINGE (**11**), ARTSCHWAGER (**1**), TUSCHNJAKOWA (**10**), and BILLINGS (**2**) have described microsporogenesis or have recorded the chromosome numbers in some members of the Chenopodiaceae.

### Materials and methods

Floral tips of *Kochia trichophylla* Staph., *K. scoparia* Schrad., *Chenopodium hybridum* L., *C. album* L., *Atriplex patula* var. *hastata* L., and *Salsola kali* L. were collected in the summer of 1933 on or near the campus of the University of Wisconsin by Dr. D. C. COOPER, Dr. K. L. MAHONY, and the writer. The identification of the species was verified by Dr. J. J. DAVIS. The material was fixed in Carnoy's solution, imbedded in paraffin, and sectioned at a thickness of 14 $\mu$. The sections were stained in Delafield's haematoxylin because of its clear differentiation of the parts of the mature pollen grain.

All drawings were made with an Abbé camera lucida at table level. A no. 4 Leitz ocular and an achromatic oil immersion N.A. 1.32 objective were used.

## Investigation

### *Kochia trichophylla*

The stamen contains rows of primary sporogenous cells which divide to form microspore mother cells (fig. 1). These cells are larger than the surrounding cells of the theca. During the maturation of the spore mother cells the nuclei of the tapetal cells undergo mitosis; ultimately each tapetal cell has two nuclei.

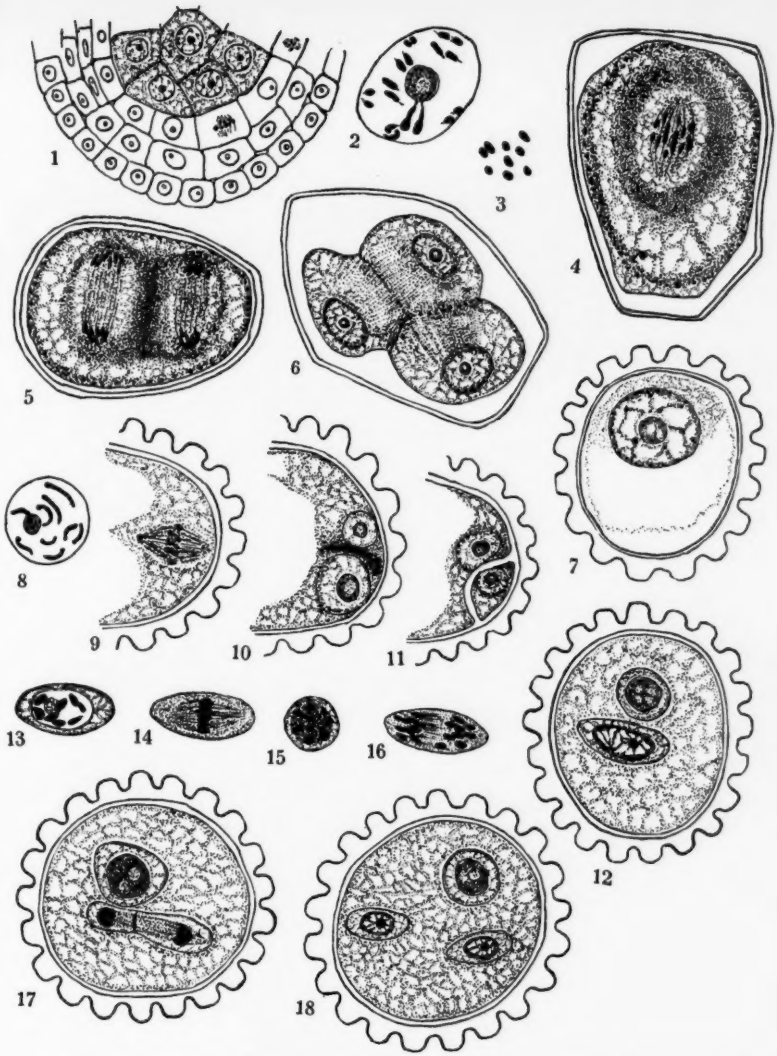
At diakinesis nine pairs of chromosomes are present in a spore mother cell (fig. 2). The paired chromosomes vary as to shape; one pair, larger than the others, appears to be attached to the nucleolus. The cell enlarges markedly during heterotypic prophases.

Figure 3 shows a polar view of nine chromosomes on the equatorial plate. Although the several pairs of chromosomes varied in size during diakinesis, they appear very similar in size on the heterotypic spindle. This spindle (fig. 4) is surrounded by a dense cytoplasmic zone. Near the periphery of the cell is a granular zone. These two zones are separated by a region containing vacuoles.

The spindles of the homoeotypic division lie side by side, either parallel to or at right angles to each other. Each spindle is surrounded by a dense cytoplasmic zone similar to that found in the heterotypic division. That region of the two cytoplasmic zones which lies between the spindles is particularly dense at the anaphase stages of nuclear division (fig. 5).

The position of the four nuclei after the completion of the homoeotypic division suggests that the homoeotypic spindles may often lie at right angles to each other (fig. 6). The nuclei are connected by the two prominent homoeotypic spindles and four less prominent spindles which are perhaps the remains of the heterotypic spindle. Thickenings appear on the fibers of each of these spindles midway between each pair of nuclei.

After the microspores have become separated they enlarge and their walls thicken, taking on the markings characteristic of the pollen grains of the Chenopodiaceae. The nucleus enlarges and




FIGS. 1–18.—*Kochia trichophylla:* fig. 1, longitudinal section through portion of anther showing microspore mother cells and mitotic figures in tapetal cells; fig. 2, late diakinesis showing nine pairs of chromosomes, one pair apparently attached to nucleolus; fig. 3, polar view of heterotypic equatorial plate showing nine chromosomes; fig. 4, heterotypic anaphase, cytoplasmic zone surrounding the spindle, and granular zone at periphery of cell; fig. 5, homoeotypic telophase showing cytoplasmic and granular zones; fig. 6, microspore formation, daughter nuclei distinct, and cell plate formation shown by thickenings on spindle fibers; fig. 7, microspore showing wall sculpturing; large vacuole present in cytoplasm; fig. 8, microspore nucleus with nine chromosomes; fig. 9, equatorial plate stage of microspore nucleus; figs. 10, 11, cell plate formation and maturation of tube and generative cells; fig. 12, young pollen grain with tube and generative cells; figs. 13–17, stages in division of generative cell; fig. 18, pollen grain with two male gamete cells within the tube cell. Fig. 1, ×1000; figs. 2–18, ×2000.

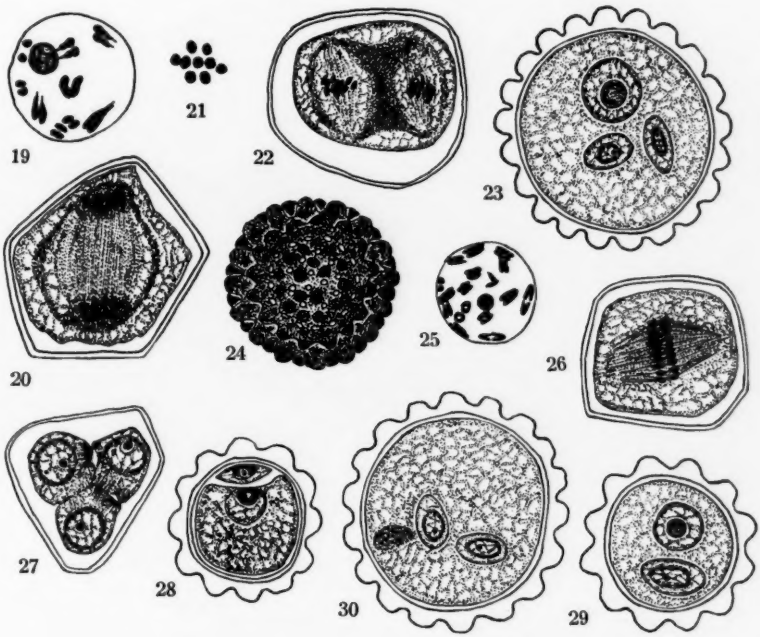
comes to lie at one side of the cell, imbedded in dense cytoplasm. A large vacuole fills most of the remainder of the space within the microspore wall. The nucleus divides, its chromatin becomes aggregated into a spireme, and later nine chromosomes can be seen (fig. 8). A spindle is now formed at right angles to the wall of the microspore, one pole being closely adjacent to this wall (fig. 9). Thickenings appear on the spindle fibers midway between the nuclei, formed as a result of the division of the microspore nucleus, and a cell plate is formed (fig. 10). As a result of this division the microspore is divided into a small generative cell and a much larger tube cell. The two cells are surrounded by thin membranes (fig. 11).

The nucleus of the tube cell is spherical; its nucleolus is large and its cytoplasm granular with very small vacuoles. The ovoid generative cell with its ovoid nucleus enlarges and becomes imbedded in the cytoplasm of the tube cell (fig. 12). The generative nucleus divides and a cell plate is formed equidistant between the two daughter nuclei separating the generative cell into male gamete cells (figs. 13–17). In the mature pollen grain the tube cell cytoplasm stains heavily whereas the cytoplasm immediately surrounding each of the male gamete nuclei stains lightly and a definite membrane seems to separate the two types of cytoplasm. The tube nucleus does not continue to enlarge whereas there is some further growth of the pollen grain (fig. 18).

### *Kochia scoparia*

Nine pairs of chromosomes are present in diakinesis stages of *K. scoparia* (fig. 19). One of the larger pairs is apparently attached to the nucleolus in a manner similar to that already described for *K. trichophylla*. Granular cytoplasmic zones surround the spindles of both the heterotypic and homoeotypic divisions, although they are not so distinct as those observed in *K. trichophylla* (figs. 20–22). The nine chromosomes seen in a polar view of the heterotypic equatorial plate are similar in size and shape (fig. 21), there being little evidence of the variations earlier found at diakinesis. The microspore mother cells in *K. scoparia* are somewhat smaller than those of *K. trichophylla*. A marked difference in the sizes of the mature pollen grains of these species was also observed, those of *K. scoparia* being the

smaller of the two. Otherwise, as seen in sectional view, they are similar. The wall of the pollen grain is so sculptured as to have




FIGS. 19–30.—Figs. 19–24, *Kochia scoparia:* fig. 19, late diakinesis, nine pairs of chromosomes present; fig. 20, heterotypic telophase showing cytoplasmic zone and slight granular zone at periphery of cell; fig. 21, polar view of heterotypic telophase (nine chromosomes present); fig. 22, homoeotypic equatorial plates with surrounding cytoplasmic zones; fig. 23, pollen grain with two male gamete cells and tube cell; fig. 24, pollen grain, surface view. Figs. 25–30, *Chenopodium hybridum:* fig. 25, late diakinesis showing 18 pairs of chromosomes; fig. 26, heterotypic equatorial plate (note absence of dense cytoplasmic zone); fig. 27, microspore formation (cell plate indicated by thickenings on spindle fibers midway between daughter nuclei); fig. 28, two-celled pollen grain; fig. 29, pollen grain with tube and generative cells; fig. 30, pollen grain with two male gamete cells and disintegrating tube nucleus. ×2000.

marked depressions both as seen in sectional (fig. 23) and in surface view (fig. 24). At the bottom of each of these depressions the wall is very thin.

*Chenopodium hybridum*

The chromosomes as seen at diakinesis are ovoid in shape and show little variation in size (fig. 25). There are 18 pairs. A distinct spindle is present during the heterotypic division and there is no evidence of cytoplasmic or granular zones such as are found in *Kochia* (fig. 26). At microspore formation the nuclei are connected by the spindle fibers of the homoeotypic and heterotypic divisions. Thickenings appear on the fibers midway between the nuclei. The cell plate splits and ingrowing septa, which are newly forming cell walls, cut across the spindle and separate the four microspores (fig. 27). Whether this is followed or accompanied by furrowing could not be determined, but it is suggested that, since osmotic changes are occurring at all times within the cell, the effect of furrowing is produced by the spore assuming a spherical shape. The splitting of the cell as seen in figure 27 would indicate that the ingrowing septa are the result rather than the cause of microspore formation. The development of the pollen grain follows much the same order as in *Kochia* (figs. 28–30).

The mature microgametophyte contains two male gamete cells and a tube nucleus that is in an advanced stage of disintegration.

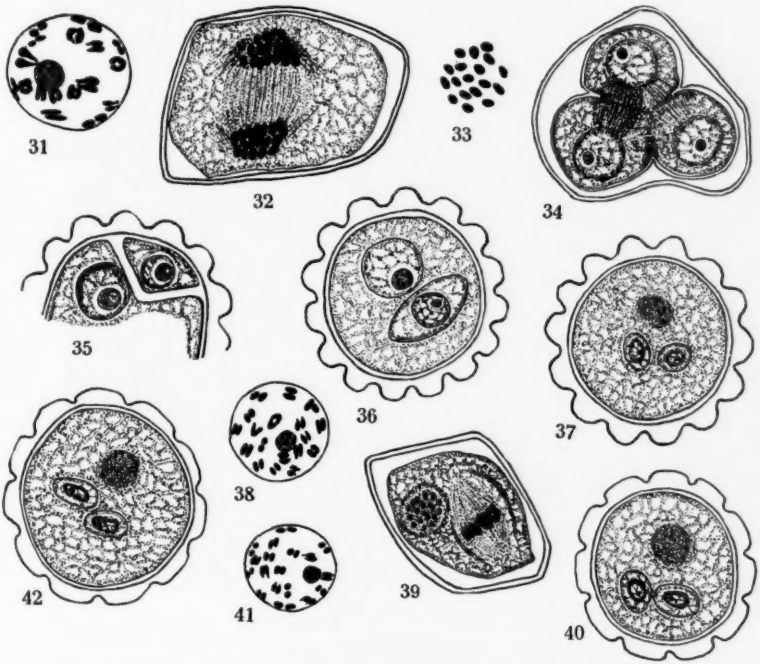
*Chenopodium album*

Eighteen pairs of irregularly shaped chromosomes are present at diakinesis in *C. album*, and as in the species of *Kochia* already described, one of the larger pairs appears to be attached to the nucleolus (fig. 31). Cytoplasmic and granular zones such as found in the meiotic divisions of *Kochia* are absent in this species (fig. 32). The chromosomes as seen in a polar view of the heterotypic telophase are 18 in number and regular in shape (fig. 33). The microspores are separated by cell plates and the development of the microgametophyte is essentially the same as that described for *C. hybridum* (figs. 34–37).

*Atriplex patula* var. *hastata*

Diakinesis shows 18 pairs of chromosomes all of which are similar in size and shape (fig. 38). The granular cytoplasmic zone is absent but there is a small zone of denser cytoplasm about each spindle (fig. 39). Eighteen chromosomes can be counted on the spindle seen

in the polar view in this figure. The mature pollen grain has two male gamete cells; the tube nucleus is in a stage of disintegration.




FIGS. 31–42.—Figs. 31–37, *Chenopodium album:* fig. 31, late diakinesis showing 18 pairs of chromosomes; fig. 32, heterotypic telophase; fig. 33, heterotypic telophase, polar view showing 18 chromosomes; fig. 34, microspore formation showing splitting of cell plate and ingrowing septa; fig. 35, portion of young two-celled pollen grain; fig. 36, pollen grain with tube and generative cells; fig. 37, pollen grain with two male gamete cells and disintegrating tube nucleus. Figs. 38–40, *Atriplex patula* var. *hastata:* fig. 38, late diakinesis showing 18 pairs of chromosomes; fig. 39, homoeotypic spindles at right angles to each other, with cytoplasmic zone present and 18 chromosomes countable on one equatorial plate; fig. 40, pollen grain with two male gamete cells and disintegrating tube nucleus. Figs. 41–42, *Salsola kali:* fig. 41, late diakinesis showing 18 pairs of chromosomes; fig. 42, pollen grain with two male gamete cells and disintegrating tube nucleus. ×2000.

The rounded portions of the pollen grain wall are considerably broader and more flattened than in the species already described (fig. 40).

*Salsola kali*

The haploid chromosome number in *Salsola kali* is 18. A long pair of chromosomes lie closely adjacent to the nucleolus at diakinesis (fig. 41). The sculpturing on the wall of the pollen grain is similar to that of *Atriplex patula* var. *hastata*, in that there are fewer depressions and the raised portions of the wall are broad and somewhat flattened (fig. 42).

## Discussion

The haploid chromosome numbers herein reported but not previously recorded, in so far as the writer is aware, are as follows: *Kochia trichophylla* 9, *K. scoparia* 9, *Salsola kali* 18, and *Atriplex patula* var. *hastata* 18. WINGE (11) likewise found 18 pairs of chromosomes in *A. patula* but only nine pairs in *A. hastata*. ROSENBERG (7) recorded about 24 as the diploid number of chromosomes in *A. hastata*. In *A. hymenelytra*, BILLINGS (2) found nine to be the haploid number in some daughter cells and ten in others.

*Chenopodium hybridum* and *C. album* each have 18 pairs of chromosomes. WINGE (11) reported nine as the haploid number in each of these species.

The presence of cytoplasm and granular zones was observed in the heterotypic and homoeotypic divisions in both *Kochia* species and in *Atriplex*. They were not found in *Chenopodium hybridum*, *C. album*, or *Salsola kali;* nor were they recorded by TUSCHNJAKOWA (10) in *Spinacea oleracea* or by ARTSCHWAGER (1) in *Beta vulgaris*. The presence of such zones, however, is not uncommon in other angiosperms.

The formation of the microspores by cell plate formation and not by furrowing, as has been described by early workers, is characteristic of *Kochia trichophylla*, *Chenopodium hybridum*, and *C. album*.

The behavior observed in the divisions of the generative cell and its nucleus to form the two male gametes suggests very definitely the formation of a cell plate.

Male cells are present in each of the species studied but are larger in *Kochia*. The tube nuclei disintegrate early and in mature pollen grains they appear to be diffusing into the surrounding cytoplasm

(figs. 30, 37, 40, 42). TUSCHNJAKOWA (10) in *Spinacea* shows the tube nucleus beginning to disintegrate whereas ARTSCHWAGER (1) makes no mention of this in *Beta*.

## Summary

1. The primary sporogenous cells in the members of the Chenopodiaceae examined divide to form several rows of microspore mother cells.

2. Prior to the nuclear divisions of the microspore mother cells the tapetal cells divide mitotically and each cell becomes binucleate.

3. The heterotypic spindle is surrounded by cytoplasmic and granular zones in *Kochia* and *Atriplex*. Such zones are not present in *Chenopodium*. This stage was not observed in *Salsola*.

4. Remains of the heterotypic and the homoeotypic spindles persist so that the tetrad nuclei are connected by two prominent spindles and four less distinct spindles.

5. Paired thickenings appear on the cytoplasmic strands midway between each of the nuclei. The cell plate splits and a wall is laid down so that ultimately four microspores are formed.

6. The microspore nucleus migrates to the periphery of the cell and there divides to form the generative and tube nuclei. By means of cell plate formation midway between these two nuclei, two cells of equal size are formed.

7. The generative cell ultimately becomes imbedded in the cytoplasm of the tube cell.

8. The generative cell divides to form two male gametes. Each gamete consists of a nucleus and a definite layer of cytoplasm delimited by a distinct membrane.

9. The tube nucleus of the mature pollen grain shows evidence of disintegration in all the species examined with the exception of the two species of *Kochia*.

10. The haploid number of chromosomes is as follows: *Kochia trichophylla* 9, *K. scoparia* 9, *Chenopodium hybridum* 18, *C. album* 18, *Atriplex patula* var. *hastata* 18, *Salsola kali* 18.

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# ORIGIN OF THE FRINGE TISSUE OF THE COTTON SEED[1]

R. G. REEVES

(WITH EIGHT FIGURES)

## Introduction

The delicate white sheath inclosing the embryo of the cotton seed has attracted attention since the cotton seed first came into prominence as a commercial product. It has been studied only incidentally, however, by those who have investigated the structure of the cotton seed.

This sheath was found by HANAUSEK (4) to consist of two tissues, the outer of which he designated as fringe cells (Franzenzellen). HANAUSEK agreed with HARZ (5) that this is the remains of the perisperm. He showed the inner portion of the sheath to be endosperm. BRETFELD (3) described the outer portion of this sheath as "dem Knospenkern hervorgegangenen Schichte." VAN ZWALUWENBURG and SCHLOTTERBECK (9), in their discussion of the entire membrane which separates the inner pigment layer from the embryo, state that it is "composed of the obliterated nucellus and endosperm, and the inner epidermis of the inner integument." WINTON (7) referred to the tissue as perisperm, thereby indicating that it is the remaining tissue of the nucellus. BALLS (1) concluded that all of the cells of the inner integument lying within the palisade layer are disorganized and that their fragmentary remains form the second pigment layer. He stated further that the inner epidermis of the inner coat (inner integument) disappears with the parenchymatous cells in marked contrast to the enormous palisade layer formed from the outer epidermis.

In a previous report which included a brief discussion of the fringe tissue, REEVES and VALLE (6) explained it as the remains of the nucellus, as HARZ, HANAUSEK, BRETFELD, WINTON, and BALLS had previously done. The tissue was accordingly designated as peri-

[1] Texas Agricultural Experiment Station, Technical Series no. 306.

sperm. Since that time, however, this tissue has been the subject of a more detailed study, and the evidence concerning its origin is now conclusive.

## Methods

While studies of comparative anatomy of seeds of malvaceous plants were in progress, several observations were made which indicated that the fringe tissue originates as the inner epidermis[2] of the inner integument. This layer of cells was therefore definitely identified in very young ovules, and followed through successively older stages to the mature condition.

The material was imbedded in paraffin and stained with Delafield's haematoxylin.

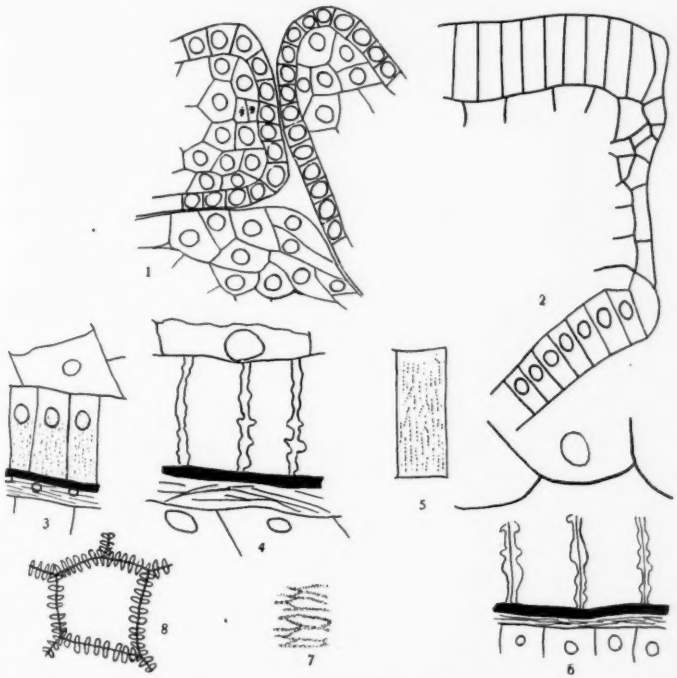
## Observations and results

When mature seeds are stored the embryo often shrinks until it becomes loose in the hull. Under such conditions the fringe tissue adheres to the endosperm and the two usually separate from the hull, remaining attached to the embryo. At maturity the fringe tissue is highly differentiated. Its cells are devoid of contents, and their walls are composed of lignin and suberin. The layer is thicker near the micropyle than elsewhere.

In ovules fixed soon after fertilization the outer layer of the inner integument was easily traced completely through the endostome on both sides of the foramen, as it appeared in sections. Here the layer of cells under observation was seen to be continuous (fig. 1). It was thus possible to identify definitely the inner epidermis of the inner integument. The epidermis was difficult to trace through the endostome except in the earliest stages, for as the ovule develops the epidermis becomes less prominent through the endostome. The identity of the epidermal layer was further determined by locating the cuticle. VAN WISSELINGH (**8**) showed that, although the cuticle between the integuments is lost rather early, that between the inner integument and the nucellus remains and even becomes thicker. In the present study, the cuticle was easily recognized just beneath the layer here regarded as the inner epidermis.

[2] The terms inner epidermis and outer epidermis are used here for convenience, it being fully understood that the integument is completely surrounded by a single epidermis.

In this early stage of development, the cells of the inner epidermis are small, dark-staining, and have large nuclei. The next significant stage shows the inner epidermis of the inner integument well on its




FIGS. 1–8.—Fig. 1, section through endostome of young cotton ovule showing epidermises of the two integuments; ×450. Fig. 2, section of slightly older inner integument at endostome showing beginning of differentiation of both epidermises; ×450. Fig. 3, fringe cells showing first signs of markings on walls; ×865. Fig. 4, longitudinal section of slightly older fringe cells showing absence of protoplasm; ×865. Fig. 5, same, surface view; ×865. Fig. 6, longitudinal section of nearly mature fringe cells showing their separation from the tissue external to themselves; ×865. Fig. 7, lateral view of markings on wall of mature fringe cell; ×1170. Fig. 8, section through mature fringe cell showing details of markings on walls; ×865.

way in development (fig. 2). The cells of both the inner and the outer epidermis elongate considerably and for a time almost equally. The cells of the inner epidermis elongate first near the micropyle;

those successively farther toward the chalaza elongate later. The cells in the region of the chalaza, however, never reach the extreme lengths of those nearer the micropyle. This accounts for the greater thickness of the fringe tissue near the micropyle. This is the layer of cells regarded by BARRITT (**2**) as inner palisade cells.

When the cells are from twice to three times as long as wide they cease to elongate. At this time their nuclei are inclined to lie in the end directed outward, and their protoplasm is still very dense.

Markings occur on the cell walls of the inner epidermis early in the development of the tissue, being first observed at about the time when the nuclei begin to migrate outward (fig. 3). They become more prominent as the seed develops, and at maturity are very conspicuous.

Shortly after the nuclei migrate outward, the cytoplasm shows signs of disintegration. When it is partially disintegrated, the nuclei break down and disappear. Long before the seed is mature, the cells of the inner epidermis become entirely devoid of contents (figs. 4, 5).

During this time there is evident resorption of cells in the parenchyma tissue of the inner integument (fig. 6). This resorption begins along the external side of the epidermis, and soon disconnects the epidermis from the remaining parenchyma. This fact explains why the fringe tissue is never found attached to the inner integument.

The peculiar markings on the walls of mature fringe cells have been briefly discussed by previous investigators. BRETFELD (**3**) described them as being nodular and wavy. HANAUSEK (**4**) stated that the tissue consists of polyhedric cells whose walls are especially fringed, highly ramified, and proliferated. VAN ZWALUWENBURG and SCHLOTTERBECK (**9**) noted that the cells of this membrane have walls thickened irregularly, rendering the outlines of the cells wavy and indistinct. VAN WISSELINGH (**8**) observed that in cross sections this tissue has radial marks upon the cell walls, and in tangential sections the marks are seen to be caused by characteristic thickenings upon the rest of the thin cell walls. Apparently the descriptions given by these investigators embody our best knowledge up to the present time of the structure of this tissue.

A slight similarity between these markings and those of the palisade cells (outer epidermis) was observed, but this similarity is only superficial. The markings of the inner epidermis are seen to be reticulate in arrangement when observed under high magnification (fig. 7), while those of the outer epidermis are parallel.

The thin areas left among the thickenings of these cell walls appear to be very similar to, if not identical with, simple pits. On close examination of well prepared sections at high magnification, they appear to be areas in which no secondary wall was laid down. Further, the thin places on the walls of adjoining cells usually coincide; that is, they are opposite each other (fig. 8). Slight variation was apparent in the details of this modification of cell wall structure. This apparent variation may be accounted for, however, by the fact that the true nature of the markings was seen only under the most favorable conditions.

Outer tangential walls were never observed, and inner tangential walls appeared to be lacking also. Whenever the tissue was separated from that adjoining on both sides, no tangential walls, either inner or outer, were ever observed. Since under natural conditions the fringe layer always separates from the inner pigment layer (which lies above it) and adheres to the layers below, the cuticle beneath this layer occupies the place of an inner tangential wall.

Of the nucellus, only the cuticle and a few fragments remain at the time of maturity. The cuticle is closely associated with and indistinguishable from the cuticle of the inner epidermis of the inner integument.

## Summary

The tissue of the cotton seed often termed perisperm is here shown to originate as the inner epidermis of the inner integument. This term, however, is properly applied to tissues which originate from the nucellus only, and its misuse in the past when applied to the cotton seed is the result of a lack of information concerning its origin. HANAUSEK'S name Franzenzellen does seem appropriate, however, and has already been translated into English as "fringe cells" (7). The names fringe cells and fringe tissue seem entirely satisfactory.

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# CERTAIN NOVELTIES IN BIDENS L. AND COREOCARPUS BENTH. (COMPOSITAE)

EARL EDWARD SHERFF

COREOCARPUS ARIZONICUS **macrophyllus** var. nov.—Folia majora, saepe 9–11.5 cm. longa, segmentis plus minusve atro-punctatis et plerumque 1.5–2 mm. latis, terminali saepe 6–8 cm. longo.

**Specimens examined:** *Dr. Edward Palmer* 294, southwestern Chihuahua, Mexico, August–November, 1885 (type, Herb. U.S. Nat.: cotypes, Herb. Gray; Herb. N.Y. Bot. Gard.).

**Bidens coreocarpoides** sp. nov.—Herba glabrata, ramosa, erecta e radice simplici tamen forsan perennis, ∓3 dm. alta, ramis tenuibus suberectis. Folia non perspicua, opposita, pinnatim 3- vel 5-partita vel summa simplicia, breviter petiolata petiolis plus minusve hispido-ciliatis 2–8 mm. longis, petiolo adjecto 1.5–3 cm. longa, segmentis rhachi similibus linearibus crassiusculis acriter induratoapiculatis 0.4–1 mm. latis. Capitula elongato-pedunculata pedunculis subcorymbose dispositis tenuissimis saepius nudis plerumque 8–12 cm. longis, radiata, pansa ad anthesin circ. 1.5–1.8 cm. lata et circ. 6–7 mm. alta. Involucrum glabrum, bracteis exterioribus circ. 8, appressis, oblongo-linearibus, apice subacuto induratis, quam interioribus oblongo-ovatis dimidio brevioribus. Flores ligulati plerumque 8, flavi, circ. 7-nervati, ligula plus minusve oblongi, apice denticulati, 7–9 mm. longi. Flores disci sub 3 mm. longi. Ovaria plana, oblongo-linearia vel cuneato-linearia, corpore ∓1.5 mm. longa, apice biaristata aristis retrorsum hamosis.

**Specimens examined:** *E. W. Nelson* and *E. A. Goldman* 7389, alt. 50–200 feet, from Cape San Lucas to San Jose del Cabo, southernmost Baja (Lower) California, Jan. 4, 1906 (type, Herb. U.S. Nat.).

In habit somewhat suggestive of *Coreocarpus arizonicus* (A. Gray) Blake.

**Coreocarpus shrevei** sp. nov.—Herba annua, simplex vel suberecte pluriramosa, glabra vel glabrata, 3–5 dm. alta, caule ramisque tenuissimis. Folia subsessilia vel petiolata petiolis tenuibus 0.5–2.5 cm. longis, petiolo adjecto 1.5–4 (rarius –5.5) cm. longa, 2–3 pinna-

tifida; segmentis ultimis linearibus vel oblongis, membranaceissimis, non punctatis, apice acuto vel obtuso mucronulatis. Capitula tenuissime pedunculata (pedunculis saepius 1–3 ad utrumque ramum, saepe 1–3-subulato-bracteolatis, saepius 2–8 cm. longis, interdum filiformibus), ad anthesin 1.3–2.5 cm. lata et 4.5–6 mm. alta; cum fructibus 6–9 mm. latis et 3.5–4.5 mm. altis. Involucrum campanulatum vel late cylindricum; bracteis oblongis vel ovatis vel obovatis, apice plus minusve acuminatis, 3.5–4.7 mm. longis. Flores ligulati 4–7 (saepius 5), rosacei (praecipue secundum ∓4 venas) vel flavidi; tubo glabro quam involucro breviore; ligula oblonga vel obovata, saepe acri-denticulata vel -emarginata, 5–10 mm. longa. Paleae lineares vel oblongae, plerumque acutae, floribus tubulosis breviores sed achaeniis longiores. Disci flores aurantiaci, 2.2–2.8 mm. longi. Achaenia iis *Coreocarpi parthenioidis* similia sed minora (exteriora corpore 2–2.5 mm. longa) et alis saepe altius incisa (dentibus saepe manifeste separatis et saepe cuneatis).

**Specimens examined:** *A. W. Anthony*, Santo Domingo, Baja California, Feb. 23 (Herb. Univ. Calif.); *T. S. Brandegee*, Magdalena Bay, Baja California, January, 1889 (Herb. N.Y. Bot. Gard.); *idem*, Purisima, Baja California, Feb. 18, 1889 (Herb. U.S. Nat.); *G. N. Collins*, *T. H. Kearney*, and *J. H. Kempton* 148, Isla Partida, Baja California, Apr. 1, 1931 (Herb. Field Mus.); *iidem* 152, *eodem loco et tempore* (Herb. U.S. Nat.); *Marcus E. Jones* 23365, Guaymas, State of Sonora, Mexico, Jan. 26, 1927 (Herb. Univ. Calif.); *idem* 24174, Laguna Mts., Baja California, Mar. 2, 1928 (Herb. Univ. Calif.); *Dr. Edward Palmer* 19, La Paz, Baja California, Jan. 20–Feb. 5, 1890 (Herb. Gray; Herb. N.Y. Bot. Gard.; Herb. U.S. Nat.); *idem* 248, Santa Agueda, Baja California, Mar. 4–6, 1890 (Herb. Gray; Herb. U.S. Nat.); *idem* 660, mountain sides, Los Angeles Bay, Baja California, Oct. 12, 1887 (Herb. Gray; Herb. N.Y. Bot. Gard.; Herb. U.S. Nat.); *idem* 795, Lagoon Head, Baja California, Mar. 6–15, 1889 (Herb. Univ. Calif., 2 sheets; Herb. Gray; Herb. N.Y. Bot. Gard.; Herb. U.S. Nat.); *C. A. Purpus* 74, alt. 1300 ft., old diggings, Calmalli, Baja California, January to March, 1898 (Herb. Univ. Calif., 2 sheets; Herb. Field Mus.; Herb. U.S. Nat.); *Dr. Forrest Shreve* 6869, at 3 miles north of Punta Prieta (N. Lat. 29°), Baja California, Feb. 17, 1935 (type, Herb. Field Mus.); *idem*

6884, at 10 miles west of Bahia de Los Angeles (N. Lat. 29°), Baja California, Feb. 20, 1935 (Herb. Field Mus.); *idem* 7058, near Santa Rosalia (N. Lat. 27°25′), Baja California, Mar. 9, 1935 (Herb. Field Mus.); *idem* 7164, at 28 miles south of Medano Blanco (N. Lat. 25°15′), Baja California, Mar. 19, 1935 (Herb. Field Mus.); *Dr. Street*, Baja California (Herb. Gray).

COREOCARPUS SHREVEI **latilobus** var. nov.—Folia principalia usque ad 8 cm. longa, bipinnatifida, segmentis latioribus ultimis saepe 7–10 mm. latis.

**Specimens examined:** *Dr. Edward Palmer* 299, Guaymas, State of Sonora, Mexico, 1887 (type, Herb. U.S. Nat.: cotypes, Herb. Gray; Herb. N.Y. Bot. Gard.; Herb. Univ. Calif.).

**Coreocarpus robustior** sp. nov.—Herba annua, ∓5 dm. alta, subglabra, valde erecto-ramosa. Folia subsessilia vel petiolata petiolis usque ad 3 cm. longis, petiolo adjecto usque ad 7 cm. longa, 1–2-pinnatifida, segmentis oblongis vel ovatis vel terminali lineari-oblongo, mucronulatis, membranaceis. Capitula tenuiter pedunculata pedunculis 2–10 cm. longis saepe 1- vel 2-bracteatis plerumque circ. 3-congregatis, ad anthesin ∓1.7 cm. lata et 6–8 mm. alta. Involucrum campanulatum, glabratum, bracteis exterioribus ovatis circ. 4 mm. longis. Flores ligulati ∓5, tubo glabro circ. 1 mm. longa, ligula oblonga vel anguste obovata ∓6 mm. longi, albi vel praecipue secundum ∓4 venas rosacei vel subpurpurei, apice vix denticulati. Paleae anguste vel late lineares, ∓4 mm. longae. Flores disci sulphurei, circ. 2.5 mm. longi. Achaenia exteriora corpore ipso 3.5–4 mm. longa et 1.3–1.8 mm. lata, alata alis sub 0.5 mm. latis nunc pectinatim incisis nunc separatim in dentes cuneatos divisis, apice saepe biaristata aristis minutis retrorsum hispidis.

**Specimens examined:** *G. N. Collins*, *T. H. Kearney*, and *J. H. Kempton* 214, Agua Verde Bay, Baja California, Apr. 3, 1931 (type, Herb. U.S. Nat.).

Differs from *C. parthenioides* Benth. in its greater height, erect-branching habit, much larger and differently shaped leaves, mostly longer peduncles, etc. From *C. shrevei* var. *latilobus*, to which it bears a resemblance in its leaves, it differs in its more robust habit, coarser involucres, larger achenes, etc.

CHICAGO NORMAL COLLEGE

# CURRENT LITERATURE

*Temperature and Living Matter* (Protoplasma-Monographien, 8th vol.). By JAN BĚLEHRÁDEK. Berlin: Gebrüder Borntraeger, 1935. Pp. x+277. figs. 70.

The scope of this volume is indicated by the titles of the chapters, which are as follows: General principles of biological temperature action; rate of biological processes at biokinetic temperatures; variation of temperature coefficients with external and internal factors; theories of temperature coefficients; chemical properties of living systems at biokinetic temperatures; variations of morphological equilibria at biokinetic temperatures; physical properties of living systems at biokinetic temperatures; freezing and frost resistance; chilling; chill-coma and death by chilling; injury by heat and heat resistance; and stimulative effects of temperature. The presentation of the animal material is somewhat more effective than is that of the plant material. One finds omissions of material that would have allowed a somewhat more balanced presentation. However, the work is a very satisfactory monograph. The general principles discussed are applicable to plant protoplasm even when the attention is centered mainly on animal behavior. The previous monographs by KANITZ (1915) and PRZIBRAM (1923) are superseded by this work of BĚLEHRÁDEK. The advances made during the last 10 years have been significant and valuable. The author index of 37 pages indicates the widespread interest among investigators in the effects of temperature upon organisms. The book will repay careful reading. It is written in English, which will be especially appreciated by American students.—C. A. SHULL.

*Structure and Reproduction of the Algae.* Vol. I. By F. E. FRITSCH. New York: Macmillan & Co., 1935. Pp. xvii+791.

The first of two volumes on the structure and reproduction of the algae has recently appeared. These are designed to present a comprehensive account of the morphology of the algae in the English language, a task for which the author is well fitted through his lifelong work with this group of plants. But it is no easy task which he has assumed on account of the diversity of forms found in the group and because of the divergence of opinion among investigators as to relationships and taxonomic treatment.

The work is designed as an introduction to the study of the algae in the widest sense, and with this purpose in mind the author has included all the holophytic Protista, as well as their non-holophytic allies. The citation of literature is very extensive and includes practically every paper of significance from 1890 through 1933. The citations are included at the end of each order treated.

The algae have been divided into eleven classes as follows: Chlorophyceae (Isokontae), Xanthophyceae (Heterokontae), Chrysophyceae, Bacillariophyceae (Diatoms), Cryptophyceae, Dinophyceae (Peridinieae), Chloromonadineae, Euglenineae, Phaeophyceae, Rhodophyceae, and Myxophyceae (Cyanophyceae). The term "phyceae" has been adopted wherever the class includes forms with an algal organization, while for those forms in which none is known the old flagellate designation is retained.

Volume I includes a general introduction and the first eight classes as listed in the preceding paragraph. In the introduction are discussed such topics as algae and Flagellata, the broad classification of the algae, the range of structure, the special features of the algal cell, and the general course of reproduction among the algae. The classes are treated by orders, according to their phylogenetic origins, and the orders are arranged by families, with practically all the known genera included.

For the most part the illustrations are judiciously selected from the works of various investigators, and clearly reproduced.

It can scarcely be hoped that all algologists will agree with the arrangement followed in this work, but the presentation of such a vast amount of information in such compass will be stimulating and helpful not only to those whose major interest lies in this field but also to those whose interest is more or less casual.—J. M. BEAL.

*A Flower Book for the Pocket.* By MACGREGOR SKENE. London: Humphrey Milford, Oxford University Press, 1935. Pp. 380. $3.00.

Often one is requested to recommend a general, reasonably simple book, readily fitting the pocket, that will aid in the identification of wild flowers. Such a book is now available for the identification of British plants. More than 800 species are given, many of them illustrated by line drawings or in full color. The illustrations are good, but one could wish that those in color were somewhat more definite and true to natural tints and shades. The keys are simple, clear, and the descriptions have been kept non-technical without sacrifice of accuracy.

A book, or more probably several books, of similar style, for the flora of the United States should prove a satisfaction to both amateur and professional botanists in America.—E. J. KRAUS.

*Primitive Land Plants.* By F. O. BOWER. London: Macmillan & Co., 1935. Pp. xiv+658. Illus. 449. $8.00.

BOWER, who is well known as an authority on the Archegoniatae, has recently published a book on the primitive land plants. Since the appearance of his Origin of a Land Flora there have been great advances in facts relating both to the living Archegoniate plants and to the fossil forms. The Rhynie plants serve to link the bryophytes with the pteridophytes, but the gap between the algae and the bryophytes remains as great today as ever. The present book is divided into

two parts. The first part includes 23 chapters presenting the known facts of the several classes of the Archegoniate forms, from the Anthocerotales through the Filicales. Free reference is made to such works as VON GOEBEL'S Organography, CAMPBELL'S Mosses and Ferns, and to BOWER'S Origin of a Land Flora and The Ferns. A comparison and summary are given at the close of the larger groups. The second part includes six chapters given to the discussion of features common to all the classes, such as alternation, embryogeny, and the spore producing members, etc. A chapter is included on the general organographic analysis, and finally a summary of results and conclusions. This work is not a new edition of Origin of a Land Flora, but is an altogether new book. Alternation of generations as demonstrated by HOFMEISTER for the "higher Cryptogamia" formed the foundation for this study of the Archegoniatae. The primary source of the material was the living Archegoniate plants compared with the facts of paleontology. The Archegoniatae are treated as an independent, evolutionary innovation on land, rather than a direct extension of the thallophytes. The "inward urge" toward increase in size, with its accompanying complexity of structure, may take effect in both the gametophyte and the sporophyte generations. The dominant haploid phase of the bryophytes is compared with the dominant diploid phase of the pteridophytes with its telomes, enations, roots, and organs of intermediate character. This work will be a source of inspiration to all who are interested in the more primitive plants from an anatomical, physiological, or organographic standpoint.—C. L. DEEVERS.

*Handbuch der Systematischen Botanik.* By R. WETTSTEIN. Leipzig and Vienna: Franz Deutticke, 1935. Pp. x+615. Illus. 354.

The second and final volume of the fourth edition of WETTSTEIN'S Handbuch der Systematischen Botanik, the first volume of which has been noticed already in these pages (BOT. GAZ. **95**:175. 1933), has recently appeared. Continuing the detailed series of specialized treatments of the Anthophyta begun in the first volume, this second volume treats the entire group of Angiospermae. Seventy-eight pages are given principally to prefatory discussions of the Angiospermae as a group and of their major subgroups. These pages deal mainly with phylogeny, relation of floral structures and methods of fructification to those of the Gymnospermae, and leading points of view regarding the systematic arrangement of the Angiospermae. They conclude with a synoptical tabulation of the more important subdivisions.

The individual families are abundantly illustrated with both drawings and photographs, the 354 *Abbildungen* containing a total of no fewer than 2083 figures, all of a high standard of excellence. Bibliographies are full and comprehensive. The typography seems as flawless as it is humanly possible to secure. Students who use the *Handbuch* for serious work will perhaps be impressed most, however, by the evidences of high scholarship and intelligent conservatism manifest throughout its pages. For these we are indebted not only to the late Dr. RICHARD WETTSTEIN, who was the principal author of the earlier editions

of this monumental work, but also to his son, Dr. FRITZ WETTSTEIN, who took the partially finished manuscript after his father's death and brought it to completion.—E. E. SHERFF.

*Gymnosperms, Structure and Evolution.* By C. J. CHAMBERLAIN. Chicago: University of Chicago Press, 1935. Pp. xi+484. figs. 397.

This recent volume is not merely a revision of the Morphology of Gymnosperms by COULTER and CHAMBERLAIN, for it is completely reorganized and rewritten. This is indicated by the illustrations alone, more than a third of them being entirely new. Fully three-fourths of the illustrations, many of which were used in his previous publications, were prepared by the author himself. CHAMBERLAIN'S drawings are always executed with superb skill and they set a high standard in the technique of botanical illustration. Through personal investigation, the author has contributed to the morphology of nearly all of the groups of gymnosperms, so that he writes with the authority of one who is thoroughly familiar with a wide range of material and who has devoted a lifetime to teaching and research in the morphology of this group of plants.

The subject is organized and treated in twenty-one chapters, of which five are devoted to the living cycads, six to the conifers, three to Gnetales, one each to the Ginkgoales, and to the fossil Cycadofilicales, Bennettitales, and Cordaitales, with chapters on phylogeny and on the alternation of generations.

The gymnosperms are plants of extreme age, reaching back at least 300 million years. The earliest records in the Paleozoic show that there are already two distinct lines included in this group, Cycadophytes and Coniferophytes. The Cycadophytes are traced back to the Cycadofilicales, the Coniferophytes to the Cordaitales. These groups in turn lead back as distinct and separate lines to the Devonian.

The Bennettitales and Cycadales were both derived independently from the Cycadofilicales, not the latter from the former, as many have supposed. Likewise the Coniferophytes include two lines, Ginkgoales and Coniferales, derived more or less independently from the Cordaitales which became extinct at the close of the Permian. Both of the latter are represented by living forms, while the Cycadophytes are represented only by the Cycadales. The Gnetales appear to be much more recent and their origin is more obscure, although some of them show a definite affinity to the Coniferales.

The chapter on Cycadofilicales presents an excellent structural interpretation of this paleobotanical material. Many of the illustrations are new and take into account the excellent restoration of a Carboniferous landscape now among the permanent exhibits of the Field Museum of Natural History. The author also presents a series of hypothetical reconstructions with diagrammatic figures of the steps in the evolution of a seed, including the seeds of the Cycadofilicales. A seed is defined as a megasporangium which retains its megaspore. Here the reviewer would wish to raise the question of whether this definition is really adequate, or whether it should not include the continued nourishment of the female

gametophyte and its embryo through the megaspore wall. However that may be, CHAMBERLAIN takes issue with SCOTT and SEWARD, who have expressed doubt (on the basis of lack of paleobotanical evidence) as to the derivation of heterosporous forms from homosporous ancestors. From a lifetime of study of the comparative morphology of living plants, especially pteridophytes and gymnosperms, CHAMBERLAIN believes that the course of evolution has been from homospory through heterospory to the seed, and ably defends this thesis. The genetic line must have been homosporous Filicales, heterosporous Filicales, Cycadofilicales.

Five chapters are devoted to the Cycadales, the group which has been CHAMBERLAIN'S special field of investigation. He has collected and observed them throughout the world in their native habitats. Among the features which are new are more detailed accounts than have been found in his earlier books of the gametophytes of *Microcycas* and of stem anatomy, also the addition of taxonomic keys to the genera, and a brief account of experiments on hybridization among cycads.

The Coniferales are treated in six chapters. Here we find much new material, including phases of general and developmental anatomy less adequately covered in the earlier books on gymnosperms. Considerable research which has been accomplished in recent years in the embryogeny of conifers has been incorporated.

The Gnetales are treated in three chapters in which the author brings together the results of many new investigations. Even in this group, the author's treatment shows evidence of considerable original research. While he has drawn freely upon the accounts of STRASBURGER, HOOKER, PEARSON, and others, he shows many new photographs and figures, including such new details as the floral development of both the male and female flowers of *Welwitschia*.

In his chapter on phylogeny, CHAMBERLAIN summarizes some of his ideas of plant evolution alluded to throughout the book, including observations and conclusions gained from his exhaustive studies during many years. Many readers might wish to have this chapter treated more fully as one feels that the author could enlarge in many places. "It seems safe to say that from the Carboniferous onward the two great lines, Cycadophytes and Coniferophytes, have been distinct. They have some common characters, but the general outline of the life-history is the same in all seed plants, and the pteridophyte structures from which the seed evolved are similar, even in lycopods and ferns. If the two great lines of gymnosperms had a common origin, it is still to be demonstrated."

In the final chapter, alternation of generations is discussed more as it appears throughout the plant kingdom rather than in the gymnosperms alone.

A complete bibliography of 719 titles is a useful feature of the book. The style of the text is excellent and is well adapted as a textbook for advanced courses. Not only because of its review and reorganization of the subject, but also because of the valuable contribution of its many new details, this book is an indispensable reference for the investigator in this field.—J. T. BUCHHOLZ.